

(19)



Europäisches Patentamt
European Patent Office
Office européen des brevets



(11)

EP 0 538 350 B1

(12)

EUROPEAN PATENT SPECIFICATION

(45) Date of publication and mention
of the grant of the patent:
25.09.1996 Bulletin 1996/39

(21) Application number: 91913007.0

(22) Date of filing: 08.07.1991

(51) Int. Cl.⁶: **B01J 2/00**, B01J 2/30,
B01J 8/00, B01J 8/10,
B01J 8/38, B01J 13/00,
B01J 20/00, B01D 39/00,
C09K 3/32, C02F 1/28

(86) International application number:
PCT/DK91/00195

(87) International publication number:
WO 92/00799 (23.01.1992 Gazette 1992/03)

(54) SUBSTANCE CARRYING CONGLOMERATE

KONGLOMERATTRÄGERMATERIAL

CONGLOMERAT DE TRANSPORT DE SUBSTANCES

(84) Designated Contracting States:
AT BE CH DE DK ES FR GB GR IT LI LU NL SE

(30) Priority: 09.07.1990 DK 1650/90

(43) Date of publication of application:
28.04.1993 Bulletin 1993/17

(60) Divisional application: 94101585.1

(73) Proprietor: UPFRONT CHROMATOGRAPHY A/S
DK-2100 Copenhagen Ø (DK)

(72) Inventors:
• LIHME, Allan, Otto, Fog
DK-3460 Birkerød (DK)
• NIELSEN, Claus, Schäfer
DK-3050 Humlebæk (DK)
• B G-HANSEN, Thorkild, Christian
DK-2900 Hellerup (DK)

(74) Representative: Christiansen, Ejvind et al
HOFMAN-BANG & BOUTARD A/S
Adelgade 15
1304 Copenhagen K (DK)

(56) References cited:
EP-A- 0 007 783 EP-A- 0 021 267
EP-A- 0 025 309 EP-A- 0 074 221
EP-A- 0 088 404 EP-A- 0 175 568
WO-A-81/02844 WO-A-87/07851
WO-A-90/14157 SE-B- 397 669

- Dialog Information Services. File 351, World Patent Index 81-91, Dialog Accession No. 89-09788/13, NIPPON STEEL CHEM KK, "Medium for culture of plants - prepd. by bonding inorganic fibre agglomerates together by combination of polymer binder and silane coupling agent", JP 1047320, A, 890221, 8913 (Basic).
- PATENT ABSTRACTS OF JAPAN, Vol. 8, No. 162, C235, Abstract of JP 59062339, publ. 1984-04-09 (TORAY ENGINEERING K.K.).

Note: Within nine months from the publication of the mention of the grant of the European patent, any person may give notice to the European Patent Office of opposition to the European patent granted. Notice of opposition shall be filed in a written reasoned statement. It shall not be deemed to have been filed until the opposition fee has been paid. (Art. 99(1) European Patent Convention).

Description

1. BACKGROUND OF THE INVENTION5 *The Technical Field*

The present invention relates to chromatographic adsorbent particles according to the preamble to claim 1; methods of their preparation; and their use as a solid phase matrix in a fluid bed reactor.

10 "Definition of Expressions"

In the present context the expression "conglomerate" is intended to designate a composite of particles, which may comprise particles of different types and sizes, held together by conglomerating agents. Conglomerates may be of various sizes, and shapes and should preferably exhibit various degrees of mechanical rigidity depending on the application. Further, conglomerates may be chemically active or may be chemically inactive under the conditions applied.

The expression "conglomerate of controlled density" is intended to designate a conglomerate or a conglomerate particle for which in particular the particles are chosen in predetermined amounts to provide a certain density of the conglomerate with respect to the liquid in which an active substance or another constituent of the conglomerate is to be used so that the floatability or sedimentation, respectively, is controlled.

Thus, chromatographic adsorbent particles according to the invention are intentionally designed with respect to the density of the medium for their particular purpose of application, including proper consideration of the influence of their sizes on their floating or sedimentation properties. In other media, e.g. during preparation or during storage under e.g. dry conditions, the chromatographic adsorbent particles may have a density different from that in the liquid medium of use.

In the present context the expression "active substance" should be taken in a very broad sense comprising agents having desired properties for their particular purpose of application, e.g. adsorbents, ligands, reagents, and natural substances covalently bound to the conglomerate of controlled density.

"Industrial Applicability"

Chromatographic adsorbent particles having covalently bound at least one active substance are used in a wide variety of applications in chromatographic processes, e.g. high performance liquid chromatography, gel filtration, ion exchange and affinity chromatography, e.g. carrying adsorbents; diagnostic processes, e.g. carrying adsorbents for blood purification, dye chromatographic processes for albumine purification; and prophylactic processes, e.g. carrying immobilized antibodies or antigens in extracorporal circulations for removal of antigens or antibodies, bacterial toxins or other toxins, and autoimmune diseases.

Prior Art Disclosure

There are numerous disclosures in the prior art concerning particles prepared from organic and inorganic materials.

However, chromatographic adsorbent particles as claimed in claim 1 have apparently never been disclosed.

"Coated Particles"

Kuraray Co., Ltd., C.A. 98:157436t discloses beads, particles, fibres, sheets, and tubes of glass, activated carbon, silica, alumina or high molecular weight substances coated with copolymers of acrylates and carboxylic acids or amines to form selective adsorbent carriers or supports for use in selective electrodes or in column chromatography.

Sakuma et al., C.A. 111:74363c, disclose glass or polymer spheres coated with hydroxyapatite for use as a stationary phase for column chromatography.

EP-A-0266580 discloses a method for coating solid particles with a hydrophilic gel preferably agarose for various separating processes in packed columns based on adsorbent groups, e.g. ion exchanging groups, hydrophobic groups, or groups with biospecificity chemically bound to a gel. Such coating may be provided by mixing hydrophilic solid particles with a gel-forming substance above the gelling temperature in which each individual particle is coated, separated from each other, and cooled below the gelling temperature, essentially to stabilize the particles against the high pressure in e.g. HPLC applications.

Generally, all of the above mentioned coated particles are provided by coating individual particles made of the same material and having the same density.

"Hollow Particles"

US 4698317 discloses hollow microspherical glass particles having open pores, and being prepared by spray thermal decomposition of a solution, in an aqueous organic solvent, wherein the water content promotes open pore formation.

US 2797201 discloses substantially spherical, hollow particles having a "thin, strong skin" being prepared by thermal treatment of droplets of a solution of a film forming material, e.g. an organic polymer such as a phenol-formaldehyde resin, and optionally further containing a "blowing agent", i.e. an agent generating gas at the elevated temperature of the thermal treatment.

GB 2151601B discloses porous hollow particles of an inorganic material and a composite material comprising such particles supporting a selected substance such as a chromatographic organic gel. The porous hollow particles may be formed by coating a fugitive core material, e.g. organic resin beads or alginate spheres, with inorganic material, and then heating to remove the fugitive core material. Further, GB 2151602B discloses closely similar particles wherein a magnetic material, such as ferric oxide, nickel oxide or cobalt oxide, is incorporated in the inorganic shell of the particle.

The 3M Corporation (USA) markets a number of types of substantially impermeable, hollow micro-spheres of siliceous material. For example synthetically manufactured soda-lime borosilicate glass microspheres marketed by 3M in a variety of size fractions. Also, permeable hollow spheres of siliceous material derived from fly-ash are provided by Fil-lite Ltd., Runcorn, England. However, none of the commercially available microspheres are conglomerates of controlled density within the meaning of the present invention.

EP-A-0021563 discloses a material suitable for thermosetting which includes a collection of hollow particles adhesively mixed with a thermosetting resin and which material may be converted by thermosetting in to a fused solid mass having a density not greater than 0.5 g/cm^3 .

"Pharmaceutical Dosage Forms"

GB-A-2196252 discloses an oral, solid, pharmaceutical dosage form comprising conventional matrix binders including starch and cellulose, or their derivatives, and a pharmaceutically acceptable weighting agent, including inorganic compounds such as salts, oxides, or hydroxides of a metal, e.g. barium sulphate or ferrous oxide, suitable for oral administration to humans and for controlled release of a pharmaceutically active ingredient into the stomach. The controlled release unit may have any chosen density from about 2 g/ml to about 6 g/ml and may in case of a conventional pellet have a size from about 1 to about 1.4 mm , and in case of a tablet a size above 10 mm . Nothing is disclosed nor suggested about non-solid i.e. permeable or porous conglomerates of controlled density within the meaning of the present invention. Furthermore, the described pharmaceutical dosage form consists of solid particles comprising a binder and a weighting agent soluble in gastric fluid which makes the pellet or tablet disintegrate shortly after ingestion.

"Fluid Bed Particles"

Generally, for a large number of applications, the active substance for binding molecules in a liquid chromatographic fluid bed process may only temporarily be available or accessible at the right places in the fluid. Thus, for chromatographic adsorbent particles carrying active substances and often showing large variations in dispersion properties, e.g. sedimentation or floatation, the active substances may be carried in an uncontrolled manner e.g. down- or upwards in relation to the liquid depending on the density of the adsorbent particle.

In fluid bed reactors partially solving the problems of packed bed columns, i.e. the problems of suspended matter clogging up the solid-phase bed which increases the back pressures and compresses the bed disturbing the flow through the bed, the adsorbent particles are carrying the active substance in a free, fluid phase by applying a flow having an opposite direction to the direction of the relative movement of the adsorbent particle. Thus, adsorbent particles having a density larger than the fluid and moving downwards due to gravity may be kept in a free, fluid phase by an upwards flow of fluid. Also, adsorbent particles having a density less than the fluid and thus moving upwards due to buoyancy may be kept in a free, fluid phase by a downwards flow of fluid.

For fluid bed liquid chromatographic processes, the density of the solid-phase adsorbent particle is very important in controlling bed properties. However, up to now, the design of solid-phase adsorbent particles has been limited by the available material.

Generally, particles may either be designed to be impermeable to the fluid, in which case the available surface area per unit volume is small; or particles may be designed to be permeable to the fluid, in which case the material chosen has to have the correct density *per se*. Unfortunately, the most interesting materials for many applications, e.g. materials such as natural and synthetic polysaccharides like agar, alginates, carrageenans, agarose, dextran, modified starches, and celluloses; synthetic organic polymers and copolymers typically based on acrylic monomers used for chromatographic purification of proteins in packed bed columns are not of suitable density *per se*. Therefore, these materials are difficult to apply in fluid bed reactors.

Certain types of organic polymers and certain types of silica based materials may be produced to provide adsorbent particles of suitable density, but such adsorbent particles may not at the same time be suitable active substances, e.g. for protein purification procedures, where such materials may provide low permeability, non-specific interactions and denature bound proteins. Further, for such polymers, it may be difficult and expensive to design derivatisation schemes for affinity chromatography media. Also, certain types of permeable silica particles have been used for fluid bed applications. However, the properties of these materials are far from optimal. Thus, the materials are instable at pH above 7, fragile to shear forces, and provide non-specific interactions.

"Particles Having Active Substances Restricted to the Surface"

US 4032407 discloses a tapered bed bioreactor applying immobilized biological catalysts or enzymatic systems on fluidizable particulate support materials consisting of coal, alumina, sand, and glass, i.e. materials heavier than the fluid, particularly an aqueous fluid.

EP-A-0175568 discloses a three phase fluidized bed bioreactor process comprising purifying effluents in a three phase fluidized bed comprising solid particles being made by mixing a binder with an inorganic material based on aluminum silicate, granulating the resulting mixture, and firing the granules to sinter them. The specific gravity of the sintered granules is adjusted to fall into a specific range from 1.2 to 2.0 by varying the mixing ratio of inorganic powdery materials based on aluminum and binders, said sintered granules having a diameter from 0.1 to 5 mm.

EP-A-0025309 discloses a downflow fluid bed bioreactor applying biota attached to carrier particles consisting of cork, wood, plastic particles, hollow glass beads or other light weight material and having a specific gravity which is less than that of a liquid sprayed onto the upper part of a fluid bed of suspended carrier particles and conducted downward through the bed.

These three disclosures describe particulate support materials to which the attachment of the active substance is restricted to the surface of the particles limiting the amount of active substance to be obtained per unit volume compared to chromatographic adsorbent particles allowing the active substance to be attached within the particle. Thus, in many applications, it is important to have specifically designed chromatographic adsorbent particles which are able to carry as large an amount of active substance per unit volume as possible, however, such chromatographic adsorbent particles are not available in the prior art.

Thus, in great many applications of active substances in liquid chromatographic fluid bed processes, there is a need for materials of controlled density carrying the active substances.

"Distribution of Liquid in a Fluid Bed"

A disadvantage of distributing an introduced liquid in a fluid bed reactor by spraying is the formation of channels in the bed by fluid rays.

EP-A-0005650 discloses an up-flow fluid bed reactor having fluidizing fluid flow distributors at the bottom thereof providing flow paths to avoid turbulence effects. Besides requiring complicated flow paths, a disadvantage of such a distributor is that it may be clogged by particulate matter.

"Removal of Oil on Water Surfaces"

US 4142969 discloses an oleospecific hydrophobic composition comprising an intimate mixture of expanded volcanic glass consisting of perlite, a cellulose fiber, and a water repellent sizing consisting of asphalt; and a method of sorbing oleaginous compounds e.g. in selectively removing oil from the surface of water. The constituents are incorporated into a homogeneous product by a wet process, dried in an oven until essentially all moisture has been removed, and then ground up into a fluffy low density material. Nothing is disclosed nor suggested about chromatographic adsorbent particles as claimed in claim 1.

2. DISCLOSURE OF THE INVENTION

(a) Chromatographic Adsorbent Particles

It is the object of the present invention to provide improved chromatographic adsorbent particles as defined in the preamble of claim 1.

Particularly, it is the object of the present invention to provide chromatographic adsorbent particles avoiding the disadvantages of known chromatographic adsorbent particles, e.g. problems of uncontrolled sedimentation or floatation of the chromatographic adsorbent particles, the poor selectivity and capacity of chromatographic adsorbent particles, and to provide the possibility of simultaneously designing and controlling the properties of the active substance and chromatographic adsorbent particles.

According to the invention this object is fulfilled by providing chromatographic adsorbent particles as claimed in claim 1.

According to the invention, chromatographic adsorbent particles may further comprise other substances such as additives, fillings, softeners, etc., and possibly comprising a suitable surface coating.

5 In its broadest aspect the density may be controlled by selecting at least two density controlling particles from a group of particles consisting of particles of very low density, particularly hollow and impermeable particles having shells of suitable material and properties; however, non-hollow particles may be chosen when appropriate; and particles of very high density, e.g. particles based on suitable heavy elements or compounds.

10 Generally, the invention provides a new type of chromatographic adsorbent particles comprising conglomerates of controlled density, selectivity, and capacity in terms of controllable interior surface areas and materials e.g. materials having specific chemical and/or mechanical properties. Thus, compared with known chromatographic adsorbent particles for fluid and packed bed reactors, chromatographic adsorbent particles according to the invention can surprisingly be designed to have a number of advantages not previously obtained.

15 According to the invention, chromatographic adsorbent particles can be designed to have a controlled density independent of the active substances and the conglomerating agents; heavy particles can be made light, and vice versa, within a wide range of particle sizes; the density can be controlled within very broad limits, e.g. the density of a known material can be controlled for a specific application; the volume percentage of the conglomerating agent can be controlled according to the application; the total size of the final chromatographic adsorbent particles can be controlled contrary to known particles having uncontrollable sizes for specific densities suitable for particular rising and falling
20 velocities. Further, chromatographic adsorbent particles according to the invention have a relative larger capacity, i.e. a larger accessible volume, compared to e.g. known impermeable chromatographic adsorbent particles. Also, in preparing such known impermeable chromatographic adsorbent particles, the active substances to be applied are limited, e.g. limited to substances that can be attached to the particle surface. Further, contrary to known chromatographic adsorbent particles having a given mechanical strength and density, the elasticity and the mechanical strength of chromatographic adsorbent particles according to the invention can be controlled independently of the density. Also, pore sizes and e.g. biocompatibility can be controlled independently of the density in order to allow access to the interior of the
25 chromatographic adsorbent particles and to avoid denaturation e.g. of proteins.

Preferred embodiments are defined in the claims 2-22.

30 (b) Methods of Preparing Chromatographic Adsorbent Particles

Preparation of chromatographic adsorbent particles according to the invention may be obtained by various methods known *per se*, e.g. block polymerisation of monomers; suspension polymerisation of monomers; block or suspension gelation of gelforming materials, e.g. by heating and cooling (e.g. of agarose) or by addition of gelation "catalysts"
35 (e.g. adding a suitable metal ion to alginates or carrageenans); block or suspension cross-linking of suitable soluble materials (e.g. cross-linking of dextrans, celluloses, or starches or gelatines, or other organic polymers with e.g. epichlorohydrine or divinyl sulfon); formation of silica polymers by acidification of silica solutions (e.g. block or suspension solutions); mixed procedures e.g. polymerisation and gelation; spraying procedures; and fluid bed coating of density controlling particles.

40 For particularly preferred embodiments according to the invention, chromatographic adsorbent particles may be obtained by cooling emulsions of density controlling particles suspended in conglomerating agents in heated oil solvents; or by suspending density controlling particles and active substance in a suitable monomer or copolymer solution followed by polymerisation.

45 "Preparation by Gelation/Polymerisation in the Emulsified State"

A method of preparing chromatographic adsorbent particles according to the invention is defined in claim 23. Preferred embodiments are defined in claims 24 and 25.

50 "Preparation by Gelation/Polymerisation in the Block State"

Another method of preparing chromatographic adsorbent particles according to the invention is defined in claim 26. Preferred embodiments are defined in claims 27 and 28.

55 For polysaccharides such as agarose and agar, i.e. materials melting at high temperatures and solidifying at low temperatures, the conglomerating means is by heating/cooling. Further, for acryl derivatives and other monomers or mixtures of these, the conglomerating means can be selected from a group consisting of:

- a) addition of polymerisation catalyst;
- b) heating;

- c) illumination with light; and
- d) irradiation with ionizing radiation.

Particularly for heavily charged polysaccharides and polymers such as alginates and carrageenans, the conglomerating means is non-covalent cross-bonding by addition of a suitable metal ion. However, for polysaccharides in general, e.g. cellulose and its derivatives, and polymers containing e.g. amino, hydroxyl, thiol, and carboxy groups, the conglomerating means is covalent cross-bonding by addition of a suitable cross-bonding agent, e.g. epichlorohydrine, divinyl sulfon, bisepoxyranes, dibromopropanol, glutaric dialdehyde, diamines, and other bifunctional agents.

Also, the above mentioned conglomerating means may be combined in specific cases such as the preparation of conglomerates of agarose-acryl-derivatives and cross-bonded mixtures of agarose and dextran.

Further, in the above mentioned block polymerisation, the segregation step of the polymer block may be obtained by methods known *per se*, e.g. by granulation and sieving.

(c) The Use of Chromatographic Adsorbent Particles

"Solid Phase Matrix in Fluid Bed Reactors"

The invention also relates to the use of chromatographic adsorbent particles according to the invention as a solid phase matrix in fluid bed reactors as claimed in claim 29.

Generally, a fluid bed reactor may comprise a vertical reactor with an inlet, an outlet, a fluid bed of particles, and a liquid. The liquid is introduced at the inlet and dispersed, optionally through a gas head in case of down-flow reactors, on the bed of particles which are suspended and fluidized by the liquid. The liquid is conducted through the bed and a pool of reacted and/or unreacted liquid is let out at the outlet.

Down-flow fluid bed reactors have liquid inlet at the top of the reactor and fluid bed particles of specific gravity less than that of the liquid.

Up-flow fluid reactors have liquid inlet at the bottom of the reactor and fluid bed particles of specific gravity larger than that of the liquid.

The suspended particles may be reactive or may carry immobilized reactive components selected for solid phase chemical or physical processes with one or more components of the fluid in procedures such as enzymatic reactions; fermentation; ion-exchange and affinity chromatography; filtration; adsorption; catalysis; immunosorption; solid-phase peptide and protein synthesis; and microbiological growth of microorganisms.

It is an object of the invention to provide the use of chromatographic adsorbent particles according to the invention in solid-phase chemical processes in continuous fluid bed reactors particularly for separation of proteins.

Preferred uses of chromatographic adsorbent particles are defined in procedures as claimed in claim 30.

"Distribution of Liquid in the Fluid Bed of a Liquid Fluid Bed Reactor"

Generally, in order to carry out solid phase chemical or physical processes in a fluid bed reactor, an even and smooth distribution of liquid in the fluid bed is desired. However, fluid bed reactors known in the art do not have means known *per se* to avoid formation of channels as well as unwanted turbulence in the particle bed.

It is another object of the present invention to provide the use of chromatographic adsorbent particles according to the invention in a fluid bed reactor for distributing a liquid in the fluid bed such that the liquid is distributed evenly and with minimal or no turbulence in the fluid bed.

The use of chromatographic adsorbent particles according to the invention for this particular purpose in a liquid down-flow fluid bed reactor and in a liquid up-flow fluid bed reactor are defined in claims 31 and 33, respectively.

According to the invention this is obtained e.g. by providing a liquid down-flow fluid bed reactor comprising a vertical reactor with an inlet, an outlet, and a fluid bed of particles, wherein the particles proximal to the liquid inlet are agitated, preferably by stirring with a mechanical stirrer which does not form a vortex, forming a turbulent zone of vigorously moving (vide our comments claim 32) particles adjoining a non-turbulent zone where the particles are in stationary fluidized state, said turbulent zone having an extent determined by the degree of agitation the value of which is selected for a given flow of liquid, viscosity, and buoyancy of the particles, within a range from a degree of agitation where the particles move but to not mix to a degree of agitation where the particles mix throughout the fluid bed.

Also, the invention provides a similar method for an up-flow fluid bed reactor in which the extent of the turbulent zone is determined based on the sedimentation of the fluid bed particles instead of the buoyancy of the particles as in case of the down-flow fluid bed reactor.

It is known that fluids and solids can be mixed by agitation. However, to the applicants' knowledge, it is new to apply agitation to fluid bed reactors for the purpose of distributing the liquid flow in the fluid bed, particularly for a down-flow reactor.

Apparently, in the art, it has been anticipated that agitation of a fluid bed generates turbulence which may result in unwanted mixing of products and reactants, and in unwanted wear of the bed particles. However, according to the invention, these advantages can be limited considerably by agitating a part of the fluid bed, particularly the part of the bed proximal to the inlet of the liquid.

Particularly, for a down-flow fluidized fluid bed reactor it has turned out that agitation of the upper part of the fluid bed divides the bed in two zones:

- i) a turbulent zone with turbulence and vigorously moving particles; and
- ii) a non-turbulent zone with no turbulence and particles in a stationary fluidized state.

The two zones adjoin with a sharp interface across which the liquid flow is evenly distributed. The position of the sharp interface is controlled by the degree of agitation which is selected for a given flow of liquid, viscosity, and buoyance /sedimentation of the particles. Thus, according to the invention, a turbulent zone provides an even distribution of liquid flow to the non-turbulent zone with minimal or no turbulence.

A number of advantages are obtained.

For chromatographic applications, the dispersion of eluant is reduced, i.e. the width of the elution band is reduced. Further, formation of channels in the fluid bed is minimized.

According to the invention, agitation can be effected by stirring the fluid bed or by any agitation means, including mechanical agitation or gas injection.

According to the invention, the fluid bed particles can be different or of the same type for both the turbulent zone and the non-turbulent zone.

In a preferred embodiment, the turbulent zone may comprise inert particles of slightly different specific gravity than the particles of the non-turbulent zone. In this case, the inert particles positioned in the turbulent zone solely participate in the distribution of liquid, and not in the solid phase processes.

Generally, compared to packed bed techniques, fluid bed techniques, e.g. to be used in fluid bed chromatography, are better suitable to large scale primary purification of proteins as the steps of centrifugation and filtration can be avoided. Thus, the fluid bed techniques can be used immediately following the production of the protein, e.g. directly applying the produced extract or fermentation liquid to fluid bed purification and conversion. Accordingly, using chromatographic adsorbent particles according to the invention in fluid bed techniques, several advantages such as the control of the density, and the choice of materials to design the chemical and/or mechanical properties of the adsorbent particles, e.g. including cheaper basic materials, are obtained.

3. BRIEF DESCRIPTION OF THE DRAWINGS

The present invention is further illustrated by reference to the examples given below and to Figures 1-6, wherein

Fig. 1A shows a 40X amplified photograph of conglomerates of agarose and glass spheres prepared according to Example 1(a);

Fig. 1B shows a 40X amplified photograph of selected spherical conglomerates of agarose and glass spheres prepared according to Example 1(a);

Fig. 2 illustrates a preferred embodiment of a fluid bed reactor;

Fig. 3 illustrates another preferred embodiment of a fluid bed reactor;

Fig. 4A and 4B show perspective sketches of another preferred embodiment of a down flow fluid bed reactor;

Fig. 5 illustrate the fluid bed particles of conglomerates according to the invention in a down flow fluid bed reactor; and

Fig. 6A-6D illustrate cross-sections along the lines VIB, VIC, VID, VIE in Fig. 5.

4. DETAILED DESCRIPTION

(a) Controlled Density of Chromatographic Adsorbent Particles

Within the present context the expression "density of chromatographic adsorbent particles" designates the density of the individual chromatographic adsorbent particles in the wet state, i.e. a state where the conglomerating agent is fully hydrated, but without any interstitial liquid between individual chromatographic adsorbent particles. This means that the liquid in which the chromatographic adsorbent particles are used are determinant for the density of the chromatographic adsorbent particles in as much as this liquid penetrates into the volume of the conglomerating agent, solvates this and fill out the pores.

This density is determinant for the tendency of the particles to float or to sediment in a given liquid.

The density of chromatographic adsorbent particles according to the invention is thus dependent on the solvated density of the conglomerating agent, the concentration of conglomerating agent, the density of the density controlling particles (impermeable to the liquid and substantially non-solvated) used to regulate the density and the concentration of these.

The density of the solvated phase, i.e. the volume occupied by the conglomerating agent and the active substance will usually be dependent on the specific application of the particles and thus not allowable to be regulated by variation of the concentration of conglomerating agent. Therefore, according to the invention the density of the chromatographic adsorbent particles is regulated by the addition of density controlling particles having a density free of choice with respect to the functionality of the chromatographic adsorbent particles and also having a final concentration in the chromatographic adsorbent particles free of choice with respect to the functionality, i.e. the functionality of the active principle within the volume of the conglomerating agent is not disturbed by the density and concentration of the density controlling particles.

A crude estimate of the final density as a function of the concentration of density controlling particles can be found by the following equation:

$$\text{Density of chromatographic adsorbent particles} = ((d_c \times v_c) + (d_b \times v_b)) / (v_c + v_b)$$

d_c = density of solvated conglomerating phase

d_b = density of density controlling particles

v_c = volume occupied by solvated conglomerating phase

v_b = volume occupied by density controlling particles.

Differences in the degree of solvation occurring in different solvents have to be corrected for. Thus, for certain conglomerating agents, e.g. heavily charged polymers for ionexchange chromatography, the degree of solvation, i.e. the volume of liquid taken up per gram dry weight, may differ with several hundred percent in fluids with different ionic strength or pH.

By way of example the density of chromatographic adsorbent particles comprising agarose as the conglomerating agent and hollow glass spheres as density controlling particles is regulated by the addition of hollow glass spheres to the liquified agarose, the amount added (for example measured as gram hollow glass spheres per ml agarose) being determinant for the density of the final chromatographic adsorbent particles.

Assuming a density of the agarose phase to be 1.0 g/ml and the volume used to be one liter (1000 ml) and the density of the hollow glass spheres to be 0.2 g/ml and the amount used to be 100 g (corresponding to 500 ml) the calculated density would be:

$$((1.0 \times 1000) + (0.2 \times 500)) / (1000 + 500) = 0.73 \text{ g/ml}$$

If only 50 g of hollow glass beads were added the calculated density would be:

$$((1.0 \times 1000) + (0.2 \times 250)) / (1000 + 250) = 0.84 \text{ g/ml}$$

If instead of the hollow glass spheres, the basic particles used were solid glass spheres with a density of 2.5 g/ml and 500 g were used to the same amount of agarose, the calculated density would be:

$$((1.0 \times 1000) + (2.5 \times 200)) / (1000 + 200) = 1.25 \text{ g/ml}$$

"Concentration of Density Controlling Particles"

Generally, the density controlling particle concentration shall be as small as possible in order to obtain as high a concentration of the active substance as possible. However, depending on the application, the density controlling particles concentration by volume is selected from a group consisting of:

- 1 - 95 %
- 1.5 - 75 %
- 5 - 50 %
- 5 - 40 %
- 5 - 30 %, most preferred.

"Dimensions of Chromatographic Adsorbent Particles"

According to the invention, optimum dimensions of the chromatographic adsorbent particles of the type according to the present invention will largely depend upon the use to which they are to be put, although limitations dictated by the nature of the material and/or by the nature of the active substance and conglomerating agent within the chromatographic adsorbent particles may also play a role.

From the point of view of achieving the greatest rate of interaction of chemical species with a given mass of conglomerate of a particular type, it will generally be advantageous that the total surface area of the chromatographic adsorbent particles is as large as possible, and thus that the size of the chromatographic adsorbent particles is as small as possible.

In preferred aspects of the chromatographic adsorbent particles according to the invention, the size of substantially all of said chromatographic adsorbent particles is within a range selected from the group consisting of:

- 1-10000 μm ,
- 1- 5000 μm ,
- 1- 4000 μm ,
- 1- 3000 μm ,
- 1- 2000 μm ,
- 1- 1000 μm ,
- 50- 500 μm .

The actual size range preferred is dependent on the actual application and the desired control of the dispersion properties, e.g. sedimentation and floatation, of the chromatographic adsorbent particles both properties being dependent on the density and the size range of the chromatographic adsorbent particles. Thus, for very fast separation flow rates chromatographic adsorbent particles of relatively low or high densities and relatively large sizes are preferred. However, large chromatographic adsorbent particles may be limited in diffusion in certain applications, e.g. when proteins have to diffuse in and out of the chromatographic adsorbent particles and interact with active substances within the chromatographic adsorbent particles.

For purification and binding of proteins and other high molecular weight substances which may diffuse slowly in the chromatographic adsorbent particles, e.g. in the conglomerating agent, the preferred size of the chromatographic adsorbent particles is within a range selected from the group consisting of:

- 1-2000 μm ,
- 10-1000 μm ,
- 50- 750 μm ,
- 100- 500 μm , most preferred.

For chromatographic adsorbent particles within the context of the present invention to be of use, for example, in chromatographic separation processes, the time-scale of the process of diffusion of the liquid phases through the chromatographic adsorbent particles, where relevant, should preferably be short in order to ensure sufficiently rapid equilibration between extra- and intraparticle phases; this time-scale will often be of the order of seconds.

(b) Density Controlling Particles and Materials

In selecting density controlling particles for use as low or high density particles according to the invention, the material of the particles depends on the purpose. Generally, the material is to be sought among certain types of natural or synthetic organic polymers, primarily synthetic organic polymers, inorganic substances and compounds, metallic elements, and alloys thereof, and non-metallic elements.

"Synthetic Organic Polymers"

Among types of synthetic organic polymers which may possibly be of interest are resins of the phenol-formaldehyde type and ABS resins, but other classes of synthetic organic polymers, such as acrylic polymers, polyamides, polyimides, polyesters, polyethers, polymeric vinyl compounds, polyalkenes and substituted derivatives thereof, as well as copolymers comprising more than one such said polymer functionality, and substituted derivatives of such copolymers, may well furnish suitable candidates.

Particularly preferred low density basic particles are hollow plastic particles.

10 "Inorganic Substances and Compounds"

From the point of view of cheapness and ready availability, in some cases it is advantageous to employ particles of inorganic material, especially since materials with the greatest mechanical rigidity are generally to be found amongst inorganic materials. Thus, material of the density controlling particles employed in the conglomerate according to the invention may comprise a member selected from the group consisting of inorganic substances and compounds, metallic elements and alloys thereof, and non-metallic elements.

In a preferred aspect, the material comprises a member selected from the group consisting of:

anhydrous forms of silicon dioxide, including amorphous silica and quartz;

metal silicates, including silicates of lithium, sodium, potassium, calcium, magnesium, aluminium and iron, and metal borosilicates, such as borosilicates of said metals, metal phosphates, including hydroxyapatite, fluorapatite, phosphorite and autunite;

metal oxides and sulfides, including magnesium, aluminium, titanium, vanadium, chromium, manganese, iron, cobalt, nickel, copper and silver oxides;

non-metal oxides, including boric oxide;

metal salts, including barium sulfate;

metallic elements, including magnesium, aluminium, titanium, vanadium, chromium, manganese, iron, cobalt, nickel, indium, copper, silver, gold, palladium, platinum, ruthenium, osmium, rhodium and iridium, and alloys of metallic elements, such as alloys formed between said metallic elements;

crystalline and amorphous forms of carbon, including graphite, carbon black and charcoal.

30 "Siliceous Glassy or Ceramic Materials"

As mentioned earlier, the prior art discloses a number of examples of hollow particles of siliceous glassy or ceramic material which may be used as low density hollow particles of chromatographic adsorbent particles according to the invention, these previously disclosed particles being obtained relatively cheaply and straightforwardly by deliberate synthesis or as a fly-ash by-product of certain combustion processes.

Accordingly, in a further preferred aspect of the invention, the material of the particles employed in chromatographic adsorbent particles of both low and high density particles according to the invention is a glass, preferably an synthetic glass comprising silicon dioxide and/or a silicate.

In yet another preferred aspect of the invention, such material is a silicon dioxide-containing material derived from fly-ash, in which case the material may be amorphous (e.g. glassy) or crystalline, or to some extent both amorphous and crystalline.

45 "Magnetic Materials"

For certain applications of a conglomerate, the material of the density controlling particles may comprise an appropriate amount of magnetic material e.g. for confining or retaining the conglomerate within a particular region of, for example, a process vessel or a chromatographic column, without the need for the incorporation of physical means of confinement or retention, such as a filter.

Thus, a further aspect of the invention provides conglomerates of density controlling particles of which the particles comprises a component selected from the group consisting of:

paramagnetic metallic elements, including iron, cobalt and nickel, and paramagnetic alloys, including alloys containing said paramagnetic metallic elements;

metal oxides, including iron(II) oxide, iron(III) oxide, cobalt(II) oxide and nickel(II) oxide;

metal salts, including cobalt(II) salts, e.g. cobalt(II) phosphate, chromium(III) salts, e.g. chromium(III) fluoride, and manganese(II) salts, e.g. manganese(II) carbonate.

"Structure of Density Controlling Particles"

Further, the material of the density controlling particles within the context of the present invention may be chemically and/or physically inhomogeneous. For example, it may have a layered structure involving one or more layers of similar or different materials, e.g. various types of siliceous materials. Alternatively, for example, it may consist of a siliceous material, such as a siliceous glassy material, containing particles or regions with a high content of a metal oxide or a metallic element chemically reactive, e.g. as a catalyst.

(c) Active substances

Concerning the active substances covalently bound to the chromatographic adsorbent particles according to the invention, this may, for example, be any type of material which is useful for a given application.

According to the invention the material of an active substance comprises a member selected from the group consisting of organic and inorganic compounds or ions, non-metallic elements, and organic polymers of biological and synthetic origin.

It is preferred that the active substance comprises a member selected from the group defined in claim 15.

"Introduction of Active Substance into the Chromatographic Adsorbent Particles"

Generally, the active substance may be introduced into the chromatographic adsorbent particles in a number of ways depending on the nature of the active substance, the conglomerating agent, and the chromatographic adsorbent particles, e.g. their pore size. Thus, both low and high molecular weight ligands may be incorporated during conglomeration either by chemical cross-linking or by copolymerisation. Further, both low and high molecular weight ligands may be chemically coupled to a conglomerating agent before or after conglomeration, or they may be coupled to precursor monomers or polymers introduced together with the conglomerating agent during the conglomeration provided the desired functions of the active substance is kept intact or may be reestablished before use. However, if the means of conglomerating damages or destroys the functioning of the active substance, the fragile active substance may be introduced after conglomeration provided the conglomerate has been designed with suitable pore sizes to allow access to its interior.

"Pore Sizes and Their Formation"

The optimum size or size-range of the through-going pores will, of course, vary very considerably, depending on the use to which the permeable chromatographic adsorbent particles is to be put. Such pore sizes are difficult to characterize quantitatively; however, in terms of the size of the molecules which are to be capable of passing through the pores, a realistic upper exclusion limit for macromolecules, notably biological macromolecules, such as proteins, will often be a molecular weight of the order of magnitude of 10^8 . The practical lower limit for pore size will generally be set by physico-chemical considerations, e.g. the detailed chemical structure of the outer part and the manner in which the outer part material dissolves or reacts during the pore-formation process.

Pore sizes may typically be formed by methods known *per se*, e.g. by simply controlling the concentration of the conglomerating agent. Thus, for agarose derivatives a larger concentration will provide a smaller pore size. However, other methods may be applied depending on the conglomerating agent and e.g. the incorporated polymers and copolymers.

"Activation or Derivatization"

In cases where the conglomerating agent may not have the properties to function as an active substance, the conglomerating agent, or agents, or polymers introduced in the conglomerate, may be derivatized to function as one or more active substances by procedures of activation or derivatisation well known *per se*. Thus, materials comprising hydroxyl, amino, amide, carboxyl or thiol groups may be activated or derivatized using various activating chemicals, e.g. chemicals such as cyanogen bromide, divinyl sulfone, epichlorohydrine, bisepoxyranes, dibromopropanol, glutaric dialdehyde, carbodiimides, anhydrides, hydrazines, periodates, benzoquinones, triazines, tosylates, tresylates, and diazonium ions.

(d) Conglomerating Agents

In selecting the conglomerating agent for use as a means of keeping the density controlling particles together and as a means for binding the active substance, the conglomerating material is to be sought among certain types of natural or synthetic organic polymers, such as defined in claim 1.

"Organic Polymers"

In one aspect of the invention the material of the conglomerating agent comprises a member selected from the group consisting of organic monomers and polymers of biological and synthetic origin, preferably as defined in claim 12.

"The Active Substance as Conglomerating Agent"

In one aspect of the invention the conglomerating agent can function as the active substance. In a further aspect, the conglomerating agent may further in a mixture comprise a member selected from the group consisting of:

natural and synthetic polynucleotides and nucleic acids, including DNA, RNA, poly-A, poly-G, poly-U, poly-C and poly-T; and

natural and synthetic polysaccharides and other carbohydrate based polymers, including agar, alginate, carrageenan, guar gum, gum arabic, gum ghatti, gum tragacanth, karaya gum, locust bean gum, xanthan gum, agaroses, celluloses, pectins, mucins, dextrans, starches and heparins,

natural and synthetic peptides and polypeptides and other amino acid based polymers, including gelatins, albumins, hemoglobulins, immunoglobulins including poly- and mono clonal antibodies, antigens, protein A, protein G, lectins, glycoproteins such as ovomucoids, and biotin binding proteins e.g. avidin and streptavidin; and

other materials used as active substance provided they can conglomerate the density controlling particles.

"Activation or Derivatization of Conglomerating Agents"

In cases where the conglomerating agent may not have the properties to function as an active substance, the conglomerating agent may be derivatized to function as one or more active substances by procedures of activation or derivatisation well known *per se*. Thus, materials comprising hydroxyl, amino, amide, carboxyl or thiol groups may be activated or derivatized using various activating chemicals, e.g. chemicals such as cyanogen bromide, divinyl sulfone, epichlorohydrine, bisepoxyranes, dibromopropanol, glutaric dialdehyde, carbodiimides, anhydrides, hydrazines, periodates, benzoquinones, triazines, tosylates, tresylates, and diazonium ions.

(e) Illustration of Chromatographic Adsorbent Particles

Fig. 1A shows a 40X amplified photograph of aspherical chromatographic adsorbent particles 10 having 1-2 mm of diameter and being prepared by distributing unicellar glass microspheres 11 in conglomerating agarose 12 according to Example 1(a).

Fig. 1B shows a 40X amplified photograph of selected spherical chromatographic adsorbent particles also prepared according to Example 1(a).

(f) Fluid Bed Reactors

"C Reactor"

Fig. 2 illustrates a cross section of a preferred embodiment of a fluid bed reactor 20 composed of a outer cylinder 21, a top lid 22 with inlet 221 and connection for a stirrer 222, and a bottom lid 23 with outlet 231. Further, an inner cylinder 24 having holes and mounted on a support 25 attached to support blocks 251 and 252 allowing passage of the fluid. Stirring is performed at a suitable rate of rotation within the inner cylinder 24 to assure a sharp lower boundary 26 of the fluid bed chromatographic adsorbent particles. Without stirring, the bed of light chromatographic adsorbent particles float against the top lid 22 and have a lower boundary 27.

"T reactor"

Fig. 3 illustrates a cross section of another preferred embodiment of a fluid bed reactor 30 similar to the reactor illustrated in Fig. 2 except that the inner cylinder 24 is replaced by an inverse funnel 34 having an upper outlet 341 and supported by a support cylinder 35 that is open upwards. Chromatographic adsorbent particles arriving below the funnel in the turbulent free volume will rise up through the upper outlet 341 while fluid flow down through the outlet 231. The stirrer is placed right below the surface 36 and stirring is performed at a suitable rate of rotation to provide a sharp lower boundary 37 of the chromatographic adsorbent particles. Without stirring, the bed of light chromatographic adsorbent particles have a lower boundary 38.

"Controlled Fluid Distribution in Fluidized Reactors"

Fig. 4A and 4B show perspective sketches of a preferred embodiment of a down flow fluid bed reactor 40.

A dc-motor 41 controlled by a variable speed control 42 provides revolutions of a stirrer 43, which in a turbulent zone A agitates the fluid bed particles to generate a turbulent flow of the fluid flowing down-ward.

A sharp interface (generally of few particle diameters) is reached at the non-turbulent zone B in which the particles are stationary and an even and smooth distribution of the fluid is obtained.

In order to adapt the conditions of agitation the length of the fluid bed column can be changed by means of interchangeable chromatographic tubes 45.

(A) "Down-Flow Fluid Bed Reactor"

Fig. 5 shows a longitudinal section of a segment of a down-flow fluid bed reactor 50 comprising a vertical cylinder 54 and a fluid bed A,B,C of particles 51,52,53 suspended in a down-flow fluidizing fluid 56 let in through an inlet at the top of the reactor vessel, the particles 51,52,53 having a specific gravity less than that of the fluid. A gas head 57 is above the surface along the line VIA-VIA.

The upper part of the fluid bed is agitated by a plate formed mechanical stirrer 55 dividing the bed into a turbulent zone A, a non-turbulent zone B, and an exit zone C.

In the turbulent zone A, the agitated fluid bed particles 51 move vigorously which generates a turbulent flow of the fluid. The turbulence decreases down the turbulent zone A. A sharp interface VIC-VIC is reached at the non-turbulent zone B in which the particles 52 are in a stationary fluidized state. Across the interface VIC-VIC, the fluid flow is distributed evenly, and a smooth fluid flow is obtained in the non-turbulent zone B.

In the exit zone C, the pooled reacted and/or unreacted fluid 57 leave the fluid bed at an interface VID-VID, where particles 53 can become separated from the fluid bed by the fluid flow.

Figs. 6A-6C show cross sections of the mixing zone A along the lines VIB-VIB, VIC-VIC, and VID-VID, respectively, of the Fig. 5. Thus, Fig. 6A shows a cross section of essentially randomly moving particles 51, and Fig. 6B & 6C show cross sections of essentially stationary fluidized particles 52 and 53.

Fig. 6D shows a cross section, along the line VIE-VIE, essentially without particles.

5. EXAMPLES

All solutions employed in the following examples are aqueous solutions unless otherwise indicated.

EXAMPLE 1

Preparation of conglomerates for chromatographic adsorbent particles according to the invention based on 3M's unicellarglass microspheres "Glass Bubbles", B28/750, C15/250, and E22/400, [soda-lime-borosilicate] having a mean density of 0.28 g/cm³, 0.15 g/cm³, and 0.22 g/cm³, respectively.

(a) Low Density Agarose Hollow Glass Spheres Conglomerated Particles (not according to the invention) - Illustration of Similar Preparation

300 ml soya bean oil was heated together with 3 ml sorbitane sesquiolate to 60°C. 5 ml 6% agarose (HSA, Litex) in water was heated and 0.5 g hollow glass spheres (3 M, B28/750) having a mean density of 0.28 g/cm³ were added under stirring. Following mixing of the agarose and glass microspheres the suspension was added to the soya bean oil under heavily stirring. The emulsion formed was stirred at about 60°C in five minutes and cooled to 20°C. The solidified agarose particles containing basic particles of hollow glass spheres were washed on a sintered glass filter with sufficient ether until all soya bean oil was removed. The conglomerate was then washed with water. The conglomerate had a low density and was floating on water.

(b) Low Density Agarose Hollow Glass Sphere Conglomerated Block Polymer Particle (not according to the invention) - Illustration of Similar Preparation

300 ml 4% agarose was prepared by heating 12 g agarose (HSA, Litex) in 300 ml water. 9 g hollow glass spheres (C15/250, 3M) was added and the mixture was stirred until a homogeneous suspension was obtained. The suspension was cooled to 60°C under steadily stirring and the fluid suspension poured on to an efficiently cooled surface. The agarose glass sphere suspension was gelled over a short period. The gel block had a homogeneous distributed content of hollow glass spheres. After cooling the gel block was blended and the granulate was sorted according to size and flow ability by means of "reverse sedimentation".

(c) Low Density Polyamide Hollow Glass Sphere Conglomerated Particles (according to the invention)

5 g acrylamide and 0.5 g N,N'-methylenebis(acrylamide) were dissolved in 100 ml 0.1 M potassiumhydrogenphosphate-HCl, pH 7.0. 3 g hollow glass spheres (C15/250, 3M) were added under stirring. Following the formation of a homogeneous suspension a catalyst of 1 g ammoniumpersulphate and 0.5 ml N,N,N',N'-tetramethylethylenediamine was added for the polymerisation. Stirring was continued until a highly viscous suspension was formed. Following polymerisation the polymer block containing hollow glass spheres was blended as described under (b).

(d) Low Density Gelatin Hollow Glass Sphere Conglomerated Particles (according to the invention) - Illustration of Density Control

Five samples of 100 ml 5% gelatin (35°C) in 0.15 M sodium chloride were added hollow glass spheres (E22/400, 3M) in increasing amounts:

- A: 0 g
- B: 2 g
- C: 5 g
- D: 20 g
- E: 27 g

After adjustment of pH to 5.5 all samples were added 2.0 ml glutaric dialdehyde (25% solution, cat.no.: 820603, Merck) under thorough stirring. After 24 hours of incubation at room temperature the polymerized matrices were disintegrated in a blender. The resulting particles were separated from fines by reverse sedimentation (for A by sedimentation as these particles were not floating). The particles were then collected on a glass filter and drained for excess water by vacuum suction on the glass filter. The wet but drained particles were then weighed and the particle volume determined by adding a known amount of liquid followed by determination of the total volume. The following particle densities were obtained:

	Measured Density:	Calculated Density:
A:	1.0 g/ml	1.00 g/ml
B:	0.9 g/ml	0.93 g/ml
C:	0.8 g/ml	0.85 g/ml
D:	0.6 g/ml	0.63 g/ml
E:	0.5 g/ml	0.57 g/ml

(e) Low Density Gelatin Hollow Glass Sphere Conglomerated Particles, and Immobilization of Horse-Radish Peroxidase (according to the invention) - Illustration of Size Range Control

1 g of horse-radish peroxidase (grade II, Kem-En-Tec, Denmark) was dissolved in a solution of 100 ml 10% gelatin (cat.no.: G-2500, Sigma) and 0.5 M sodium chloride (35°C). 10 g hollow glass spheres (B28/750, 3M) were added under stirring. After adjustment of pH to 5.5, 2 ml glutaric dialdehyde (25% solution, cat.no.: 820603, Merck) was added with thorough stirring. The resulting gel was incubated at room temperature for 2 hours and then disintegrated in a blender. The floating particles were separated from fines and non-floating particles by inverse sedimentation. The yield of wet, packed particles was approx. 120 ml. The size range was determined to be from about 200 to about 500 µm in diameter.

(f) Low Density Agar-Gelatin Glass Sphere Conglomerated Particles I

2 g agar (Bacto-agar, Difco), and 3 g gelatin (cat.no.: G-2500, Sigma) was dissolved in 100 ml 0.15 M sodium chloride by brief heating to the boiling point. After cooling to about 56 °C 10 g hollow glass beads (B28/750, 3M) were added. pH was adjusted to 4.0 with 5 M acetic acid followed by the addition of 2 ml glutaric dialdehyde (25% solution, Cat. No. 820603, Merck) with thorough stirring. The resulting polymer block was cooled to room temperature and incubated for 24 hours followed by disintegration in a blender.

Floating particles were separated from fines and non-floating particles by inverse sedimentation followed by collection of the floating particles on a glass filter.

The yield of floating conglomerate particles was 95 ml packed wet particles.

5 (g) *Low Density Agar-Gelatin Glass Sphere Conglomerated Particles II*

2 g agar (Bacto-agar, Gibco) and 3 g gelatin (cat.no.: G-2500, Sigma) was dissolved in 100 ml 0.15 M sodium chloride by brief heating to the boiling point. After cooling to about 56 °C 10 g hollow glass beads (B28/750, 3M) were added. The suspension was then cooled by pouring it onto an ice-cold glassplate. The resulting gel block was incubated for 24 hours at 4 °C followed by disintegration by blending in ice-water. The conglomerate floating gel-particles were separated from non-floating particles by inverse sedimentation and then collected on a glass filter. The yield was 105 ml of packed, wet particles.

The particles were then suspended in 200 ml 0.1 M potassium phosphate buffer pH 6.5 and crosslinked for two hours by addition of 10 ml glutaric dialdehyde (25% solution, 820603, Merck).

15 (h) *Low Density Chitosan Glass Sphere Conglomerated Particles*

A 4% solution of chitosan (Cat. No.: 22741, Fluka) was prepared by heating 12 g chitosan in 300 ml 10% v/v acetic acid. The viscous solution was cooled to about 40 °C followed by addition of 20 g hollow glass beads (B28/750, 3M). 3 ml glutaric dialdehyde was added (25% solution, 820603, Merck) with thorough stirring. The resulting polymer block was incubated for 24 hours at room temperature followed by disintegration in a blender.

The conglomerate floating gel-particles were separated from non-floating particles by inverse sedimentation in 0.1 M sodium chloride and then collected on a glass filter. The yield was 400 ml of packed, wet particles with a diameter from about 200 µm to about 800 µm.

25 (i) *Vinyltriethoxysilan Coated Glass Spheres and Polyamide Conglomerated Particles*

(A) "Coating of the glass spheres"

30 75 g (dry) hollow glass spheres (C15/250, 3M) were mixed with 500 ml 1% vinyltriethoxysilan solution in 0.1 M acetic acid and the suspension was stirred for one hour. The vinyltriethoxysilan solution was removed by filtration on a glass filter.

(B) "Conglomerating acrylamide and glass spheres"

35 1.5 g N,N'-methylenebisacrylamide was dissolved in 10 ml ethanol and mixed with 8.5 g acrylamide dissolved in 90 ml water. 15 g vinyltriethoxysilan coated glass spheres, from (A), was added under stirring. 0.5 g ammoniumpersulphate and 0.5 ml N,N,N',N'-tetramethylethylenediamine was added as polymerisation catalysts after a homogeneous suspension was reached. The stirring was continued until the polymer block was formed. The polymer block was subsequently blended as described in Example 1(b) and "fines" were removed by "inverse sedimentation". This procedure resulted in approx. 100 ml low density conglomerate.

EXAMPLE 2

45 *Chemical Derivatisation of Low Density Agarose Glass Sphere Conglomerated Particles Prepared According to Example 1 (a)(b) - Illustration of Similar Use*

10 g (dried, wet weight) agarose conglomerate spheres containing hollow glass spheres from Example 1 were suspended in 100 ml 0.5 M potassiumphosphate/sodiumhydroxide pH 11.4. 10 ml divinyl sulfone and 50 mg sodiumborohydride were added under stirring. The suspension was stirred at room temperature for three hours and the spheres were washed with water on a glass filter. The spheres were then activated chemically (i.e. a method out of many possibilities) and were ready for coupling of other substances. As an example mercaptoethanol was coupled for salt-dependent chromatography: The spheres were reacted with 5% mercaptoethanol in water that had been titrated to pH 9.5 with 1 M sodiumhydroxide for 3 hours at room temperature.

55 The spheres were then washed thoroughly with distilled water and were ready to be used as chromatographic adsorbent particles in purification of proteins using salt-dependent chromatography.

EXAMPLE 3

Purification of Human Immunoglobulin from Untreated Blood Using Chromatographic Adsorbent Particles Prepared According to Example 2 - Illustration of Similar Use

100 g (dried, wet weight) divinylsulfon and mercaptoethanol treated agarose conglomerate spheres equilibrated with and suspended in 50 ml 0.75 M ammoniumsulfate were placed in a cylindrical glass column with an inner diameter of 5 cm and length of 10 cm. The glass column was sealed at the top and bottom using unscrewing plastic caps. The bottom lit had an outlet with a tube piping in the middle while the top lit had a corresponding inlet and a mechanical stirrer. The mechanical stirrer provides stirring through a air tight collar for stirring the conglomerate spheres contained in the column. The stirring propeller was designed to avoid fluid flow that carries the agarose conglomerate spheres down to the outlet in the bottom column. 2 l unfiltered and not centrifugated human blood (i.e. outdated blood from a blood bank) having been added ammoniumsulfate to a final concentration of 0.75 M is lead through the column from the top with a flow of 10 ml/min under stirring with the abovementioned stirrer (i.e. to avoid the formation of channels through the fluid bed). 2000 ml of 0.75 M ammoniumsulfate was added at the same flow rate for washing non-bound proteins and particulates. Finally, the bound proteins were eluted from the conglomerate spheres by leading 500 ml of 0.1 M sodium chloride through the column.

About 5 g human immunoglobulin was eluted in the sodium chloride fraction. Qualitative analysis showed a high purity of immunoglobulin having a very small contamination of albumin (< 1%).

A corresponding purification of immunoglobulins with divinylsulfon and mercaptoethanol treated agarose spheres without hollow glass spheres was not possible in a traditionally packed column because of clogging of the column by the red blood cells and other sticky materials in blood plasma.

EXAMPLE 4

Immunsorption Using Chromatographic Adsorbent Particles Prepared According to Example 2 - Illustration of Similar Use

Agarose conglomerate spheres containing 4% agarose and produced as described in Example 1 were activated with divinylsulfon as described in Example 2.

10 g (drained, wet weight) activated gel was coupled to rabbit immunoglobulin by incubation of the gel over night with 20 ml rabbit immunoglobulin solution (10 mg immunoglobulin/ml in 0.1 M sodiumhydrogencarbonate/sodiumhydroxide buffer, pH 8.6 and 5% w/v polyethylenglycol MW 20,000). Excess active groups were blocked by incubation of the gel with 0.5 M ethanolamine/HCl, pH 9.0 for three hours. The gel was coupled with more than 80% of the added rabbit immunoglobulin.

The floating conglomerate spheres having rabbit immunoglobulin attached could then be applied in an apparatus corresponding to the one in Example 3 for adsorption of anti-bodies against rabbit immunoglobulin from untreated serum of previously pure rabbit immunoglobulin immunized goats. The separated antibody was of a purity and activity corresponding to that obtained with conventionally packed columns using filtered and centrifuged antiserum.

EXAMPLE 5

Preparation of Ion Exchange Conglomerates.

(a) *Cation Exchange Conglomerates. Conglomeration of polyacrylic acid/acrylamide/N,N'-methylen-bis(acrylamide) and hollow glass spheres.*

300 ml distilled water was added to 25 ml acrylic acid, 100 ml ethanol, 10 g N,N'-methylen-bis(acrylamide), 25 g acrylamide, 2 g ammoniumpersulfate, 25 g hollow glass spheres (B28/750, 3M) and 2 ml N,N,N',N'-tetramethylethylenediamine. The mixture was stirred until a homogeneous suspension was achieved and then titrated to pH 8.5 with 5 M sodiumhydroxide under steadily stirring. Stirring was continued until polymerisation of the suspension occurred.

Following polymerisation the block was blended as described in Example 1 (b) and "fines" were separated by means of "inverse sedimentation". Following a thoroughly wash of the particles with water, 0.1 M HCl and 0.1 M NaCl, the content of carboxyl groups in the gel was determined to be about 250 μ mol per g drained wet gel by simple titration.

(b) Conglomeration of Acrylic Acid/acrylamide/N,N'-methylenbisacrylamide and Vinyltriethoxysilan Coated Hollow Glass Spheres

60 g (dry) hollow glass spheres (C15/250, 3M), 40 ml acrylic acid, 32 g acrylamide, 8 g N,N'-methylenbisacrylamide and 5 ml vinyltriethoxysilane was added to 300 ml distilled water. The mixture was stirred for one hour and brought to pH 7 with cold 27.4 % sodium hydroxide. 1 g ammoniumpersulphate and 1 ml N,N,N',N'-tetramethylethylenediamine was added as polymerisation catalysts and stirring was continued until the polymer block was formed. The polymer block was subsequently blended as described in Example 1(b) and "fines" were removed by "inverse sedimentation". In a batch protein binding assay, pH 9, 50 mM TRIS/HCl, 1 g of drained wet conglomerate was able to bind 96% of 190 mg offered lysozyme.

(c) Conglomeration of Acrylic Acid/methacrylamide/N,N'-methylenbisacrylamide and Vinyltriethoxysilan Coated Hollow Glass Spheres

Following the procedure described in Example 5(b), this ion exchange conglomerate was prepared as by using methacrylamide in exchange for acrylamide. In a batch protein binding assay, pH 9, 50 mM TRIS/HCl, 1 g of the resulting drained wet conglomerate was able to bind 92% of 190 mg offered lysozyme.

(d) Conglomeration of Acrylic Acid/methacrylamide/N,N'-methylenbisacrylamide and Vinyltriethoxysilan Coated Hollow Glass Spheres

Following the procedure described in Example 5(c), this ion exchange conglomerate was prepared as by using only 20 ml acrylic acid, 16 g methacrylamide and 4 g N,N'-methylenbisacrylamide, giving the conglomerate a lower dry weight content allowing larger proteins to diffuse in and out of the conglomerate. In a batch protein binding assay, pH 9, 50 mM TRIS/HCl, 1 g of conglomerate was able to bind 92% of 190 mg offered lysozyme.

EXAMPLE 6

Immobilized Enzyme. Immobilization of Glucose Oxidase on Chromatographic Adsorbent Particles Prepared According to Example 2 - Illustration of Similar Use

10 g divinylsulfon activated agarose conglomerate spheres from Example 2 were mixed with 20 ml of a solution of glucose oxidase from *Aspergillus niger* (10 mg/ml in 1 M potassiumhydrogenphosphate/sodiumhydroxide buffer, pH 10.5). The mixture was left for three hours and the uncoupled glucose oxidase was washed out of the spheres by 1 M sodium chloride.

The enzyme coupled conglomerate spheres showed glucose oxydase activity with glucose as a substrate. The development of hydrogen peroxide was detected as a brown colouring of the gel and solution by coupling the reaction with peroxidase (horse-radish peroxidase) oxidation of orthophenylen diamine.

EXAMPLE 7

Chromatographic Adsorbent Particles Comprising Immobilized N-acetylglucosamine for the Separation of Wheat Germ Agglutinin - Illustration for Similar Use

Conglomerate spheres containing 4% agarose and produced as described in Example 1 (b) were activated with divinyl sulfon as described in Example 2. 10 g (dried, wet weight) of the activated gel was coupled to N-acetylglucosamine by incubating the gel over night with 20 ml 0.5 M potassiumphosphate/sodiumhydroxide buffer pH 11.5 containing 50 mg N-acetylglucosamine per ml. Following incubation the excess of active vinyl groups were blocked by 5% mercaptoethanol titrated to pH 9.5 by sodium hydroxide. The gel was washed thoroughly with 1 M sodium chloride. The binding capacity for wheat germ agglutinin was larger than 10 mg lectin per ml gel.

EXAMPLE 8

(a) High Density Acrylic Acid Copolymer Solid Glass Sphere Conglomerated Particles

To 300 ml distilled water was added 40 ml acrylic acid, 28 g acrylamide, 12 g N,N'-methylenbisacrylamide, 5 ml vinyltriethoxysilane and 245 g solid glass spheres (0.075-0.15 mm, Fryma, Switzerland). The suspension was stirred for one hour and then adjusted to pH 7 with cold 27.4% sodium hydroxide. 1 g ammoniumpersulfate and 1 ml N,N,N',N'-tetramethylethylenediamine was added as polymerization catalysts and the stirring was continued until a polymer block

was formed. The polymer block was subsequently disintegrated in a blender followed by repeated sedimentation to remove fines. This procedure gave about 800 ml conglomerated particles with a density of 1.3 g/ml. In a batch protein binding assay (50 mM Tris/HCl pH 9.0) 1 g of wet but drained conglomerated particles was able to bind 61% of 190 mg offered lysozyme from hens egg white.

(b) High Density Gelatin Solid Glass Sphere Conglomerated Particles (according to the invention) - Illustration of Density Control

Four samples of 100 ml 5% gelatin in 0.15 M sodium chloride (35°C) were added solid glass spheres (0.075-.15 mm, Fryma, Switzerland) with a density of 2.5 g/ml in increasing amounts:

- A: 10 g
- B: 50 g
- C: 100 g
- D: 200 g

After adjustment of pH to 5.5 all samples were added 2.0 ml glutaric dialdehyde (25% solution, Cat. No.: 820603, Merck) under thorough stirring. After 24 hours of incubation at room temperature the polymerized matrices were disintegrated in a blender. The resulting particles were separated from fines by sedimentation. The particles were then collected on a glass filter and drained for excess water by vacuum suction on the glass filter. The wet but drained particles were then weighed and the particle volume determined by adding a known amount of liquid followed by determination of the total volume. The following particle densities were obtained:

	Measured Density:	Calculated Density:
A:	1.1 g/ml	1.06 g/ml
B:	1.3 g/ml	1.25 g/ml
C:	1.5 g/ml	1.43 g/ml
D:	1.7 g/ml	1.67 g/ml

Claims

1. Chromatographic adsorbent particles having covalently bound at least one active substance for binding molecules in a liquid chromatographic fluid bed process;
said adsorbent particles being constituted by a porous composite material having pores allowing access to the interior of the composite material of said molecules;
CHARACTERIZED in

(a) that the porous composite material consists of a conglomerate having controlled density; said conglomerate consisting of:

(i) at least two density controlling particles selected from the group consisting of low density particles having a density providing floatation and high density particles having a density providing sedimentation of the conglomerate in said liquid; and

(ii) a matrix formed by consolidating at least one conglomerating agent selected from the group consisting of natural and synthetic organic monomers and polymers;

said at least two density controlling particles being dispersed in said matrix;

(b) that the size range of the adsorbent particles is controlled;

(c) that said density and said size range are selected to provide desired floatation/sedimentation properties of said adsorbent particles in the liquid in said fluid bed process; and

(d) that the at least one active substance is covalently bound to said matrix:

with the proviso that when said at least two density controlling particles are of amorphous silica, quartz, or glass, then said conglomerating agent does not consist of natural and synthetic polysaccharides and other carbohydrate based polymers.

2. Particles according to claim 1, CHARACTERIZED in that said density controlling particles are impermeable to the fluid.
3. Particles according to claim 1, CHARACTERIZED in that the low density particles are hollow.
4. Particles according to claim 1, CHARACTERIZED in that the high density particles are solid.
5. Particles according to claim 1, CHARACTERIZED in that the density controlling particles constitute from 1 to 95 %, generally from 1.5 to 75 %, particularly from 5 to 50 %, preferably from 5 to 40 %, and most preferably from 5 to 30 %, by volume of the conglomerate.
6. Particles according to claim 1, CHARACTERIZED in that the density controlling particles are made of a material selected from the group consisting of natural and synthetic organic polymers, inorganic substances and compounds, metallic elements and alloys thereof, and non-metallic elements.
7. Particles according to claim 6, CHARACTERIZED in that the synthetic organic polymers are selected from the group consisting of:
 - resins of the phenol-formaldehyde type;
 - ABS resins;
 - polyamides, polyimides, polyesters, polyethers, polymeric vinyl compounds, polyalkenes, and substituted derivatives thereof; and
 - copolymers comprising more than one such said polymers, and substituted derivatives of such copolymers.
8. Particles according to claim 6, CHARACTERIZED in that the inorganic substances and compounds, metallic elements and alloys thereof, and non-metallic elements are selected from the group consisting of:
 - anhydrous forms of silicon dioxide, including amorphous silica and quartz;
 - metal silicates, including silicates of lithium, sodium, potassium, calcium, magnesium, aluminium and iron, and metal borosilicates, such as borosilicates of said metals, metal phosphates, including hydroxyapatite, fluorapatite, phosphorite and autunite;
 - metal oxides and sulfides, including magnesium, aluminium, titanium, vanadium, chromium, manganese, iron, cobalt, nickel, copper and silver oxides;
 - non-metal oxides, including boric oxide;
 - metal salts, including barium sulfate;
 - metallic elements, including magnesium, aluminium, titanium, vanadium, chromium, manganese, iron, cobalt, nickel, indium, copper, silver, gold, palladium, platinum, ruthenium, osmium, rhodium and iridium, and alloys of metallic elements, such as alloys formed between said metallic elements; and
 - crystalline and amorphous forms of carbon, including graphite, carbon black and charcoal.
9. Particles according to claim 1, CHARACTERIZED in that the at least two density controlling particles are made of amorphous silica, quartz, or glass.
10. Particles according to claim 1, CHARACTERIZED in that the low density particles consist of hollow particles, or unicellular glass micro spheres.
11. Particles according to claim 1, CHARACTERIZED in that the high density particles consist of glass particles, preferably glass micro spheres.
12. Particles according to claim 1, CHARACTERIZED in that the at least one conglomerating agent is made of natural or synthetic organic monomers and polymers selected from the group consisting of:

a) natural and synthetic polysaccharides and other carbohydrate based polymers, including agar, alginate, carrageenan, guar gum, gum arabic, gum ghatti, gum tragacanth, karaya gum, locust bean gum, xanthan gum, agaroses, celluloses, pectins, mucins, dextrans, starches, heparins, chitosans, hydroxy starches, hydroxypro-

pyl starches, carboxymethyl starches, hydroxyethyl celluloses, hydroxypropyl celluloses, and carboxymethyl celluloses;

b) synthetic organic polymers and monomers resulting in polymers, including acrylic polymers, polyamides, polyimides, polyesters, polyethers, polymeric vinyl compounds, polyalkenes, and substituted derivatives thereof, as well as copolymers comprising more than one such organic polymer functionality, and substituted derivatives thereof; and

c) mixtures of these.

13. Particles according to claim 1, CHARACTERIZED in that the at least one conglomerating agent is agarose.

14. Particles according to claim 1, CHARACTERIZED in that the active substance comprises a material or mixtures of materials selected from the group consisting of:

organic and inorganic compounds or ions, non-metallic elements, and organic polymers of biological and synthetic origin.

15. Particles according to claim 1, CHARACTERIZED in that the active substance comprises a member selected from the group consisting of:

ligands known *per se* in the field of chromatography, charged species for ion exchange chromatography, proteins, dyes, enzyme inhibitors, specific ligands for specific proteins, biotin for purification of avidin and other biotin binding proteins, carbohydrates for purification of lectins or glycosidases, protein A, chelates, iminodiacetic acid, amino acids, arginine, lysine, and histidine, sulfated polymers, heparins, benzhydroxamic acid, hydrophobic ligands, hydrocarbon groups such as phenyl, thiophilic ligands, divinyl sulfone activated substances coupled with mercaptoethanol, 4-hydroxy-pyridine, 3-hydroxy-pyridine, or 2-hydroxy-pyridine;

natural and synthetic polynucleotides and nucleic acids, including DNA, RNA, poly-A, poly-G, poly-U, poly-C and poly-T;

natural and synthetic polysaccharides and other carbohydrate based polymers, including agar, alginate, carrageenan, guar gum, gum arabic, gum ghatti, gum tragacanth, karaya gum, locust bean gum, xanthan gum, agaroses, celluloses, pectins, mucins, dextrans, starches and heparins;

natural and synthetic peptides and polypeptides and other amino acid based polymers, including gelatins, albumins, hemoglobulins, immunoglobulins including poly- and mono clonal antibodies, antigenes, protein G, lectins, glycoproteins such as ovomucoids, biotin binding proteins, avidin and streptavidin, enzymes, proteases, and protease inhibitors; and

mixtures of these.

16. Particles according to claim 1, CHARACTERIZED in that the active substance is covalently bound to the adsorbent particles by means of activation or derivatisation agents activating or derivatizing the conglomerating agent, or the conglomerated particles.

17. Particles according to claim 16, CHARACTERIZED in that the activation or derivatisation agents are selected from a group consisting of:

cyanogen bromide, divinyl sulfone, epichlorohydrine, bisepoxyranes, dibromopropanol, glutaric dialdehyde, carbodiimides, anhydrides, hydrazines, periodates, benzoquinones, triazines, tosylates, tresylates, and diazonium ions.

18. Particles according to claim 1, CHARACTERIZED in that the density is from 0.1 to 15, particularly from 0.1 to 5, and preferably from 0.2 to 2.

19. Particles according to claim 1, CHARACTERIZED in that the density is from 1 to 15, particularly from 1.1 to 5, and preferably from 1.1 to 2.

20. Particles according to claim 1, CHARACTERIZED in that the density is from 0.1 to 1, generally from 0.2 to 0.95, particularly from 0.3 to 0.8, and preferably from 0.5 to 0.75.

21. Particles according to claim 1, CHARACTERIZED in that their size is within a range selected from the group consisting of:

1-10000 μm ,

1-5000 μm ,

1-4000 μm ,
 1-3000 μm ,
 1-2000 μm ,
 1-1000 μm ,
 50-500 μm .

5

22. Particles according to claim 21, particularly for purification and binding proteins and other high molecular weight substances, CHARACTERIZED in that their size is within a range selected from the groups consisting of:

10

1-2000 μm ,
 10-1000 μm ,
 50-750 μm ,
 100-500 μm , most preferred.

23. A method of preparing chromatographic adsorbent particles as defined in claim 1, CHARACTERIZED by

15

a) mixing particles selected from the group consisting of low density particles having a density providing floatation, and high density particles having a density providing sedimentation of the conglomerate in the liquid with at least one conglomerating agent selected from the group consisting of natural and synthetic organic monomers and polymers;

20

and optionally heating said mixture;

b) emulsifying said mixture in a suitable solvent;

25

c) consolidating said conglomerating agent by a suitable means such as gelation by heating/cooling, polymerisation of monomer or monomer mixtures, and non-covalent or covalent cross-bonding; and

d) isolating and washing the consolidated conglomerated particles.

24. A method according to claim 23, CHARACTERIZED in that the isolated and washed conglomerate particles are sorted according to size and flow ability.

30

25. A method according to claim 23 or 24, CHARACTERIZED in that the conglomerating agent or the conglomerate particles are activated or derivatized.

35 26. A method of preparing chromatographic adsorbent particles as defined in claim 1, CHARACTERIZED by

a) mixing particles selected from the group consisting of low density particles having a density providing floatation, and high density particles having a density providing sedimentation of the conglomerate in the liquid with at least one conglomerating agent selected from the group consisting of natural and synthetic monomers and polymers;

40

and optionally heating said mixture;

b) consolidating said conglomerating agent by a suitable means such as gelation by heating/cooling, polymerisation of monomer or monomer mixtures, and non-covalent or covalent cross-bonding;

45

c) disintegrating the block of conglomerate;

d) segregating and washing the consolidated conglomerated particles.

50 27. A method according to claim 26, CHARACTERIZED in that the segregated and washed conglomerate particles are sorted according to size and flow ability.

28. A method according to claim 26 or 27, CHARACTERIZED in that the conglomerating agent or the conglomerate particles are activated or derivatized.

55

29. The use of chromatographic adsorbent particles according to claim 1 as a solid phase matrix in a fluid bed reactor.

30. The use according to claim 29 in a procedure selected from a group consisting of:
 chromatographic procedures applying non-packed columns including liquid chromatography, ion-exchange

chromatography, and biospecific affinity chromatography such as immunosorption and protein A chromatography, and group specific affinity chromatography such as hydrophobic, thiophilic, dye, lectin, and metal chelate chromatography.

- 5 31. The use according to claim 29 or 30, CHARACTERIZED in that the fluid bed reactor is a liquid down-flow fluid bed reactor comprising a vertical reactor vessel with an inlet, an outlet, a fluid bed of said chromatographic adsorbent particles, and agitation means; said agitation means being located near or in the fluid bed proximal to the liquid inlet.
- 10 32. The use according to claim 31 for distributing the liquid in the fluid bed, CHARACTERIZED in
- a) that the chromatographic adsorbent particles and liquid proximal to the liquid inlet are agitated to divide the fluid bed into
- 15 i) a turbulent zone having vigorously moving particles, and
- ii) a non-turbulent zone;
- said non-turbulent zone adjoining said turbulent zone; and
- 20 b) that the extent of said turbulent zone is determined by a degree of agitation selected within a range from
- i) a degree of agitation providing turbulence only in the uppermost part of the fluid bed,
- 25 ii) to a degree of agitation providing turbulence of the particles throughout the fluid bed.
- 30 33. The use according to claim 29 or 30, CHARACTERIZED in that the fluid bed reactor is a liquid up-flow fluid bed reactor comprising a vertical reactor vessel with an inlet, an outlet, a fluid bed of said chromatographic adsorbent particles, and agitation means; said agitation means being located near or in the fluid bed proximal to the liquid inlet.
34. The use according to claim 33 for distributing the liquid in the fluid bed, CHARACTERIZED in
- 35 a) that the chromatographic adsorbent particles and liquid proximal to the liquid inlet are agitated to divide the fluid bed into
- i) a turbulent zone having vigorously moving particles, and
- ii) a non-turbulent zone;
- 40 said non-turbulent zone adjoining said turbulent zone; and
- b) that the extent of said turbulent zone is determined by a degree of agitation selected within a range from
- 45 i) a degree of agitation providing turbulence only in the lower-most part of the fluid bed,
- ii) to a degree of agitation providing turbulence of the particles throughout the fluid bed.

Patentansprüche

- 50 1. Adsorptionsteilchen für Chromatographie mit mindestens einer kovalent gebundenen aktiven Substanz zum Binden von Molekülen in einem Flüssigchromatographie-Fließbettverfahren;
- wobei die Adsorptionsteilchen aus einem porösen Verbundmaterial mit Poren aufgebaut sind, die Zugang der Moleküle zu dem Inneren des Verbundmaterials zulassen;
- dadurch gekennzeichnet, daß**
- 55 a) das poröse Verbundmaterial aus einem Konglomerat mit einer gesteuerten Dichte besteht und das Konglomerat aus:

- (i) mindestens zwei dichtesteuernden Teilchen, ausgewählt aus der Gruppe, die aus Teilchen mit niedriger Dichte mit einer Dichte, die ein Schwimmen bereitstellt, und Teilchen mit hoher Dichte mit einer Dichte, die eine Sedimentation des Konglomerats in der Flüssigkeit bereitstellt, besteht; und
- (ii) einer Matrix, die durch sich Verfestigen von mindestens einem Konglomerationsmittel gebildet ist, das aus der Gruppe ausgewählt ist, die aus natürlichen und synthetischen organischen Monomeren und Polymeren besteht,

besteht, wobei die mindestens zwei dichtesteuernden Teilchen in der Matrix dispergiert sind;

(b) der Größenbereich der Adsorptionsteilchen gesteuert ist;

(c) die Dichte und der Größenbereich so ausgewählt sind, daß erwünschte Schwimm-/Sedimentationseigenschaften der Adsorptionsteilchen in der Flüssigkeit in dem Fließbettverfahren bereitgestellt werden; und

(d) die mindestens eine aktive Substanz kovalent an die Matrix gebunden ist,

mit der Maßgabe, daß wenn die mindestens zwei dichtesteuernden Teilchen aus amorphem Siliziumdioxid, Quarz oder Glas sind, das Konglomerationsmittel nicht aus natürlichen und synthetischen Polysacchariden und anderen Polymeren auf Kohlenhydratbasis besteht.

2. Teilchen nach Anspruch 1,

dadurch gekennzeichnet, daß

die die Dichte steuernden Teilchen für die Flüssigkeit undurchdringlich sind.

3. Teilchen nach Anspruch 1,

dadurch gekennzeichnet, daß

die Teilchen mit niedriger Dichte hohl sind.

4. Teilchen nach Anspruch 1,

dadurch gekennzeichnet, daß

die Teilchen mit hoher Dichte massiv sind.

5. Teilchen nach Anspruch 1,

dadurch gekennzeichnet, daß

die die Dichte steuernden Teilchen 1 bis 95 Vol.-%, allgemein 1,5 bis 75 Vol.-%, insbesondere 5 bis 50 Vol.-%, bevorzugt 5 bis 40 Vol.-% und am bevorzugtesten 5 bis 30 Vol.-% des Konglomerats darstellen.

6. Teilchen nach Anspruch 1,

dadurch gekennzeichnet, daß

die die Dichte steuernden Teilchen aus einem Material hergestellt sind, das aus der Gruppe ausgewählt ist, die aus natürlichen und synthetischen organischen Polymeren, anorganischen Substanzen und Verbindungen, metallischen Elementen und ihren Legierungen und nichtmetallischen Elementen besteht.

7. Teilchen nach Anspruch 6,

dadurch gekennzeichnet, daß

die synthetischen organischen Polymere aus der Gruppe ausgewählt sind, die aus:

Harzen vom Phenol-Formaldehyd-Typ;

ABS-Harzen;

Polyamiden, Polyimiden, Polyestern, Polyethern, polymeren Vinylverbindungen, Polyalkenen und substituierten Derivaten dieser Verbindungen; und

Copolymeren, die mehr als eines dieser Polymere umfassen, und substituierten Derivaten von solchen Copolymeren besteht.

8. Teilchen nach Anspruch 6,

dadurch gekennzeichnet, daß

die anorganischen Substanzen und Verbindungen, metallischen Elemente und ihre Legierungen und nichtmetallischen Elemente aus der Gruppe ausgewählt sind, die aus:

wasserfreien Formen von Siliziumdioxid, umfassend amorphes Siliziumdioxid und Quarz;

Metallsilikaten, umfassend Silikate aus Lithium, Natrium, Kalium, Kalzium, Magnesium, Aluminium und

Eisen- und Metall-Borosilikaten, wie beispielsweise Borosilikaten aus diesen Metallen, Metallphosphaten, umfassend Hydroxylapatit, Fluorapatit, Phosphorit und Autunit;

Metalloxiden und -sulfiden, umfassend Magnesium-, Aluminium-, Titan-, Vanadium-, Chrom-, Mangan-, Eisen-, Kobalt-, Nickel-, Kupfer- und Silberoxide;

Nichtmetalloxiden, umfassend Boroxid;

Metallsalzen, umfassend Bariumsulfat;

metallischen Elementen, umfassend Magnesium, Aluminium, Titan, Vanadium, Chrom, Mangan, Eisen, Kobalt, Nickel, Indium, Kupfer, Silber, Gold, Palladium, Platin, Ruthenium, Osmium, Rhodium und Iridium, und Legierungen aus metallischen Elementen, wie beispielsweise zwischen den metallischen Elementen gebildeten Legierungen; und

kristallinen und amorphen Formen von Kohlenstoff, umfassend Graphit, Ruß und künstliche Kohle besteht.

9. Teilchen nach Anspruch 1,

dadurch gekennzeichnet, daß

die mindestens zwei dichtesteuernden Teilchen aus amorphem Siliziumdioxid, Quarz oder Glas hergestellt sind.

10. Teilchen nach Anspruch 1,

dadurch gekennzeichnet, daß

die Teilchen mit niedriger Dichte aus hohlen Teilchen oder einzelligen Mikrogaskugeln bestehen.

11. Teilchen nach Anspruch 1,

dadurch gekennzeichnet, daß

die Teilchen mit hoher Dichte aus Glasteilchen, bevorzugt Mikrogaskugeln bestehen.

12. Teilchen nach Anspruch 1,

dadurch gekennzeichnet, daß

das mindestens eine Konglomerationsmittel aus natürlichen oder synthetischen organischen Monomeren oder Polymeren hergestellt ist, die aus der Gruppe ausgewählt sind, die aus:

a) natürlichen und synthetischen Polysacchariden und anderen Polymeren auf Kohlenhydratbasis, umfassend Agar, Alginat, Karrageen, Guar Gum, Gummiarabikum, Ghatti Gum, Traganth-Gummi, Karaya-Gummi, Johannisbrot-Gum, Xanthanlösung, Agarosen, Cellulosen, Pektinen, Mucinen, Dextranen, Stärken, Heparinen, Chitosanen, Hydroxy-Stärken, Hydroxypropyl-Stärken, Carboxymethyl-Stärken, Hydroxyethyl-Cellulosen, Hydroxypropyl-Cellulosen und Carboxymethyl-Cellulosen;

b) synthetischen organischen Polymeren und Monomeren, die zu Polymeren führen, umfassend Acrylpolymer, Polyamide, Polyimide, Polyester, Polyether, polymere Vinylverbindungen, Polyalkene und substituierte Derivate dieser Verbindungen, ebenso wie Copolymere, die mehr als eine solche organische Polymerfunktionalität umfassen, und substituierte Derivate dieser Verbindungen; und

c) Mischungen aus diesen besteht.

13. Teilchen nach Anspruch 1,

dadurch gekennzeichnet, daß

das mindestens eine Konglomerationsmittel Agarose ist.

14. Teilchen nach Anspruch 1,

dadurch gekennzeichnet, daß

die aktive Substanz ein Material oder Mischungen aus Materialien umfaßt, die aus der Gruppe ausgewählt sind, die aus:

organischen und anorganischen Verbindungen oder Ionen, nichtmetallischen Elementen und organischen Polymeren von biologischem und synthetischem Ursprung besteht.

15. Teilchen nach Anspruch 1,

dadurch gekennzeichnet, daß

die aktive Substanz einen Bestandteil umfaßt, der aus der Gruppe ausgewählt ist, die aus:

Liganden, die per se im Gebiet der Chromatographie bekannt sind, geladenen Spezies für Ionenaustauschchromatographie, Proteinen, Farbstoffen, Enzyminhibitoren, spezifischen Liganden für spezifische Proteine, Biotin zur Reinigung von Avidin und anderen biotinbindenden Proteinen, Kohlenhydraten zur Reinigung von Lektinen

oder Glykosidasen, Protein A, Gelatine, Iminodiessigsäure, Aminosäuren, Arginin, Lysin und Histidin, sulfatisierten Polymeren, Heparinen, Benzhydroxamsäure, hydrophoben Liganden, Kohlenwasserstoffgruppen wie Phenyl, thiophilen Liganden, mit Divinylsulfon aktivierten Substanzen, gekoppelt mit Mercaptoethanol, 4-Hydroxy-Pyridin, 3-Hydroxy-Pyridin oder 2-Hydroxy-Pyridin;

5 natürlichen und synthetischen Polynucleotiden und Nucleinsäuren, umfassend DNA, RNA, poly-A, poly-G, poly-U, poly-C und poly-T;

natürlichen und synthetischen Polysacchariden und anderen Polymeren auf Kohlenhydratbasis, umfassend Agar, Alginat, Karrageen, Guar Gum, Gummiarabikum, Ghatti Gum, Tragant-Gummi, Karaya-Gummi, Johannisbrot-Gum, Xanthanlösung, Agarosen, Cellulosen, Pektinen, Mucinen, Dextranen, Stärken und Heparinen;

10 natürlichen und synthetischen Peptiden und Polypeptiden und anderen Polymeren auf Aminosäurebasis, umfassend Gelatine, Albumine, Hämoglobuline, Immunoglobuline, umfassend poly- und monoklonale Antikörper, Antigenen, Protein G, Lektinen, Glycoproteinen wie beispielsweise Ovomucoide, biotinbindenden Proteinen, Avidin und Streptavidin, Enzymen, Proteasen und Proteaseinhibitoren; und

Mischungen aus diesen besteht.

15 16. Teilchen nach Anspruch 1,

dadurch gekennzeichnet, daß

die aktive Substanz über Aktivierungs- oder Derivatisierungsmittel, die das Konglomerationsmittel oder die konglomerierten Teilchen aktivieren oder derivatisieren, kovalent an die Adsorptionsteilchen gebunden ist.

20 17. Teilchen nach Anspruch 16,

dadurch gekennzeichnet, daß

die Aktivierungs- oder Derivatisierungsmittel aus einer Gruppe ausgewählt sind, die aus:

25 Cyanogenbromid, Divinylsulfon, Epichlorhydrin, Bisepoxyranen, Dibrompropanol, Glutardialdehyd, Carbodiimiden, Anhydriden, Hydrazinen, Perjodaten, Benzochinonen, Triazinen, Tosylaten, Tresylaten und Diazonium-Ionen besteht.

18. Teilchen nach Anspruch 1,

dadurch gekennzeichnet, daß

30 die Dichte von 0,1 bis 15, insbesondere von 0,1 bis 5 und bevorzugt von 0,2 bis 2 ist.

19. Teilchen nach Anspruch 1,

dadurch gekennzeichnet, daß

35 die Dichte von 1 bis 15, insbesondere von 1,1 bis 5 und bevorzugt von 1,1 bis 2 ist.

20. Teilchen nach Anspruch 1,

dadurch gekennzeichnet, daß

die Dichte von 0,1 bis 1, allgemein von 0,2 bis 0,95, insbesondere von 0,3 bis 0,8 und bevorzugt von 0,5 bis 0,75 ist.

40 21. Teilchen nach Ansprüchen 1,

dadurch gekennzeichnet, daß

ihre Größe in einem Bereich liegt, der aus der Gruppe ausgewählt ist, die aus:

1 bis 10 000 μm ,

1 bis 5 000 μm ,

45 1 bis 4 000 μm ,

1 bis 3 000 μm ,

1 bis 2 000 μm ,

1 bis 1 000 μm ,

50 50 bis 500 μm

besteht.

22. Teilchen nach Anspruch 21, insbesondere zur Reinigung und zum Binden von Proteinen und anderen Substanzen mit hohem Molekulargewicht,

dadurch gekennzeichnet, daß

55 ihre Größe in einem Bereich liegt, der aus den Gruppen ausgewählt ist, die aus:

1 bis 2 000 μm ,

10 bis 1 000 μm ,

50 bis 750 μm ,

100 bis 500 μm , am bevorzugtesten, bestehen.

23. Verfahren zur Herstellung von Adsorptionsteilchen zur Chromatographie nach Anspruch 1,
gekennzeichnet durch

a) das Vermischen von Teilchen, die aus der Gruppe ausgewählt sind, die aus Teilchen mit niedriger Dichte mit einer Dichte, die ein Schwimmen bereitstellt, und Teilchen mit hoher Dichte mit einer Dichte, die eine Sedimentation des Konglomerats in der Flüssigkeit bereitstellt, besteht, mit mindestens einem Konglomerationsmittel, das aus der Gruppe ausgewählt ist, die aus natürlichen und synthetischen organischen Monomeren und Polymeren besteht;
und gegebenenfalls Erhitzen der Mischung;

b) Emulgieren der Mischung in einem geeigneten Lösungsmittel;

c) Verfestigen des Konglomerationsmittels durch ein geeignetes Mittel wie beispielsweise Gelierung durch Erhitzen/Abkühlen, Polymerisation des Monomers oder der Monomermischungen, und nicht-kovalentes oder kovalentes Verbinden; und

d) Isolieren und Waschen der verfestigten konglomerierten Teilchen.

24. Verfahren nach Anspruch 23,
dadurch gekennzeichnet, daß
die isolierten und gewaschenen Konglomeratteilchen nach Größe und Fließfähigkeit sortiert werden.

25. Verfahren nach Anspruch 23 oder 24,
dadurch gekennzeichnet, daß
das Konglomerationsmittel oder die Konglomeratteilchen aktiviert oder derivatisiert werden.

26. Verfahren zur Herstellung von Adsorptionsteilchen für die Chromatographie nach Anspruch 1,
gekennzeichnet durch

a) das Vermischen von Teilchen, die aus der Gruppe ausgewählt sind, die aus Teilchen mit niedriger Dichte mit einer Dichte, die ein Schwimmen bereitstellt, und Teilchen mit hoher Dichte mit einer Dichte, die eine Sedimentation des Konglomerats in der Flüssigkeit bereitstellt, besteht, mit mindestens einem Konglomerationsmittel, das aus der Gruppe ausgewählt ist, die aus natürlichen und synthetischen Monomeren und Polymeren besteht;
und gegebenenfalls Erhitzen der Mischung;

b) Verfestigen des Konglomerationsmittels durch ein geeignetes Mittel wie beispielsweise Gelierung durch Erhitzen/Abkühlen, Polymerisation des Monomers oder der Monomermischungen, und nicht-kovalentes oder kovalentes Verbinden; und

c) Entmischen des Konglomeratblocks

d) Segregation und Waschen der verfestigten konglomerierten Teilchen.

27. Verfahren nach Anspruch 26,
dadurch gekennzeichnet, daß
die segregierten und gewaschenen Konglomeratteilchen nach Größe und Fließfähigkeit sortiert werden.

28. Verfahren nach Anspruch 26 oder 27,
dadurch gekennzeichnet, daß
das Konglomerationsmittel oder die Konglomeratteilchen aktiviert oder derivatisiert werden.

29. Verwendung der Adsorptionsteilchen für Chromatographie nach Anspruch 1 als eine Festphasenmatrix in einem Fließbettreaktor.

30. Verwendung nach Anspruch 29 in einem Verfahren, das aus der Gruppe ausgewählt ist, die aus:

Chromatographieverfahren unter Anwendung von nicht-Füllkörpersäulen, umfassend Flüssigchromatographie, Ionenaustauscher-Chromatographie und Chromatographie unter Ausnutzung der biospezifischen Affinität wie beispielsweise Immunosorption und Protein-A-Chromatographie, und Chromatographie unter Ausnutzung der gruppenspezifischen Affinität wie beispielsweise hydrophober, thiophiler, Farbstoff-, Lektin- und Metallchelat-Chromatographie besteht.

31. Verwendung nach Anspruch 29 oder 30,

dadurch gekennzeichnet, daß

der Fließbettreaktor ein im Herabfluß betriebener Flüssigkeits-Fließbettreaktor ist, der ein vertikales Reaktorgefäß mit einem Einlaß, einem Auslaß, einem Fließbett aus den Adsorptionsteilchen für Chromatographie, und Rührmittel umfaßt, wobei sich die Rührmittel in der Nähe von oder in dem Fließbett nahe bei dem Flüssigkeitseinlaß befinden.

32. Verwendung nach Anspruch 31 zum Verteilen der Flüssigkeit in dem Fließbett,
dadurch gekennzeichnet, daß

a) die Adsorptionsteilchen für Chromatographie und die Flüssigkeit in der Nähe des Flüssigkeitseinlasses gerührt werden, wobei das Fließbett in

(i) eine turbulente Zone mit sich heftig bewegenden Teilchen und

(ii) eine nicht-turbulente Zone geteilt wird;

wobei die nicht-turbulente Zone an die turbulente Zone angrenzt; und

b) die Ausdehnung der turbulenten Zone durch den Grad des Rührens bestimmt wird, der innerhalb eines Bereichs von

i) einem Rührgrad, der Turbulenz nur in dem obersten Teil des Fließbetts bereitstellt,

ii) bis zu einem Rührgrad, der Turbulenz der Teilchen im gesamten Fließbett bereitstellt,

ausgewählt ist.

33. Verwendung nach Anspruch 29 oder 30,

dadurch gekennzeichnet, daß

der Fließbettreaktor ein im Herauf Fluß betriebener Flüssigkeits-Fließbettreaktor ist, der ein vertikales Reaktorgefäß mit einem Einlaß, einem Auslaß, einem Fließbett aus den Adsorptionsteilchen für Chromatographie, und Rührmittel umfaßt, wobei sich die Rührmittel in der Nähe von oder in dem Fließbett nahe bei dem Flüssigkeitseinlaß befinden.

34. Verwendung nach Anspruch 33 zum Verteilen der Flüssigkeit in dem Fließbett,
dadurch gekennzeichnet, daß

a) die Adsorptionsteilchen für Chromatographie und die Flüssigkeit in der Nähe des Flüssigkeitseinlasses gerührt werden, wobei das Fließbett in

(i) eine turbulente Zone mit sich heftig bewegenden Teilchen und

(ii) eine nicht-turbulente Zone geteilt wird;

wobei die nicht-turbulente Zone an die turbulente Zone angrenzt; und

b) die Ausdehnung der turbulenten Zone durch den Grad des Rührens bestimmt wird, der innerhalb eines Bereichs von

i) einem Rührgrad, der Turbulenz nur in dem untersten Teil des Fließbetts bereitstellt,

ii) bis zu einem Rührgrad, Turbulenz der Teilchen im gesamten Fließbett bereitstellt,

ausgewählt ist.

5 Revendications

1. Particules d'adsorbant chromatographique ayant au moins une substance active liée de façon covalente pour fixer des molécules dans un procédé chromatographique de liquide à lit fluidisé ; lesdites particules d'adsorbant étant constituées par une matière composite poreuse ayant des pores laissant l'accès desdites molécules à l'intérieur de la matière composite ; caractérisées

(a) en ce que la matière composite poreuse est constituée d'un conglomerat ayant une densité contrôlée ; ledit conglomerat étant constitué de :

- (i) au moins deux particules contrôlant la densité, choisies dans le groupe constitué des particules basse densité ayant une densité fournissant une flottation et des particules haute densité ayant une densité fournissant une sédimentation du conglomerat dans ledit liquide ; et
- (ii) une matrice formée en consolidant au moins un agent conglomerant choisi dans le groupe constitué des monomères et polymères organiques naturels et synthétiques ;

- lesdites deux particules contrôlant la densité au moins étant dispersées dans ladite matrice ;
- (b) en ce que la gamme de taille des particules d'adsorbant est contrôlée ;
- (c) en ce que ladite densité et ladite gamme de taille sont choisies pour fournir les propriétés de flottation/sédimentation souhaitées desdites particules d'adsorbant dans le liquide dans ledit procédé à lit fluidisé ; et
- (d) en ce que la substance active au moins est liée de façon covalente à ladite matrice ;

avec la condition que lorsque lesdites deux particules contrôlant la densité au moins sont en silice amorphe, quartz ou verre, alors ledit agent conglomerant n'est pas constitué de polysaccharides et autres polymères à base de glucide, naturels et synthétiques.

2. Particules selon la revendication 1, caractérisées en ce que lesdites particules contrôlant la densité sont imperméables au fluide.
3. Particules selon l'une quelconque des revendications 1 ou 2, caractérisées en ce que les particules basse densité sont creuses.
4. Particules selon la revendication 1, caractérisées en ce que les particules haute densité sont pleines.
5. Particules selon la revendication 1, caractérisées en ce que les particules contrôlant la densité constituent de 1 à 95 %, généralement de 1,5 à 75 %, en particulier de 5 à 50 %, de préférence de 5 à 40 %, et de toute préférence de 5 à 30 % en volume par rapport au conglomerat.
6. Particules selon la revendication 1, caractérisées en ce que les particules contrôlant la densité sont constituées d'une matière choisie dans le groupe constitué des polymères organiques naturels et synthétiques, des substances et composés inorganiques, des éléments métalliques et alliages de ceux-ci, et des éléments non métalliques.
7. Particules selon la revendication 6, caractérisées en ce que les polymères organiques synthétiques sont choisis dans le groupe constitué :
 - des résines du type phenol-formaldéhyde ;
 - des résines ABS ;
 - des polyamides, polyimides, polyesters, polyéthers, composés vinyliques polymères, polyalkylènes et dérivés substitués de ceux-ci ; et
 - des copolymères comprenant plus d'un desdits tels polymères, et des dérivés substitués de tels copolymères.
8. Particules selon la revendication 6, caractérisées en ce que les substances et composés inorganiques, les éléments métalliques et alliages de ceux-ci, et les éléments non métalliques sont choisis dans le groupe constitué :
 - des formes anhydres du dioxyde de silicium, incluant la silice amorphe et le quartz ;
 - des silicates de métal, incluant les silicates de lithium, sodium, potassium, calcium, magnésium, aluminium

et fer, et des borosilicates de silice tels que les borosilicates desdits métaux, des phosphates de métal, incluant l'hydroxyapatite, la fluorapatite, la phosphorite et l'autunite ;

des oxydes et sulfures de métal, incluant les oxydes de magnésium, aluminium, titane, vanadium, chrome, manganèse, fer, cobalt, nickel, cuivre et argent ;

des oxydes de non-métal, incluant l'oxyde borique ;

des sels de métal, incluant le sulfate de baryum ;

des éléments métalliques, incluant le magnésium, l'aluminium, le titane, le vanadium, le chrome, le manganèse, le fer, le cobalt, le nickel, l'indium, le cuivre, l'argent, l'or, le palladium, le platine, le ruthénium, l'osmium, le rhodium et l'iridium, et des alliages d'éléments métalliques, tels que les alliages formés entre lesdits éléments métalliques ; et

des formes cristallines et amorphes du carbone, incluant le graphite, le noir de carbone et le charbon.

9. Particules selon la revendication 1, caractérisées en ce que les deux particules contrôlant la densité au moins sont faites de silice amorphe, quartz ou verre.

10. Particules selon la revendication 1, caractérisées en ce que les particules basse densité sont constituées de particules creuses ou de microsphères en verre unicellulaires.

11. Particules selon la revendication 1, caractérisées en ce que les particules haute densité sont constituées de particules de verre, de préférence des microsphères en verre.

12. Particules selon la revendication 1, caractérisées en ce que l'agent agglomérant au moins est constitué de monomères et polymères organiques naturels ou synthétiques choisis dans le groupe constitué :

a) des polysaccharides et autres polymères à base de glucide, naturels et synthétiques, incluant la gélose, un alginat, une carraghénine, la gomme de guar, la gomme arabique, la gomme ghatti, la gomme adragante, la gomme karaya, la gomme de caroube, la gomme xanthane, les agaroses, les celluloses, les pectines, les mucines, les dextrans, les amidons, les héparines, les chitosanes, les hydroxyamidons, les amidons hydroxypropylés, les amidons carboxyméthylés, les hydroxyéthylcelluloses, les hydroxypropylcelluloses et les carboxyméthylcelluloses ;

b) des polymères et monomères organiques synthétiques formant des polymères, incluant les polymères acryliques, les polyamides, polyimides, polyesters, polyéthers, composés vinyliques polymères, polyalkylènes et les dérivés substitués de ceux-ci, ainsi que les copolymères comprenant plus d'une telle fonctionnalité polymère organique, et les dérivés substitués de ceux-ci ; et

c) des mélanges de ceux-ci.

13. Particules selon la revendication 1, caractérisées en ce que l'agent agglomérant au moins est l'agarose.

14. Particules selon la revendication 1, caractérisées en ce que la substance active comprend une matière ou des mélanges de matières choisies dans le groupe constitué :

des composés ou ions organiques et inorganiques,

des éléments non métalliques, et

des polymères organiques d'origine biologique et synthétique.

15. Particules selon la revendication 1, caractérisées en ce que la substance active comprend un élément choisi dans le groupe constitué :

des ligands connus d'eux-mêmes dans le domaine de la chromatographie, des espèces chargées pour la chromatographie par échange d'ions, des protéines, des colorants, des inhibiteurs d'enzyme, des ligands spécifiques pour des protéines spécifiques, la biotine pour purifier l'avidine et d'autres protéines fixant la biotine, les glucides pour purifier les lectines ou les glycosidases, la protéine A, des chélates, l'acide iminodiacétique, des acides aminés, l'arginine, la lysine et l'histidine, des polymères sulfatés, des héparines, l'acide benzhydroxamique, des ligands hydrophobes, des groupes hydrocarbonés tels que le radical phényle, des ligands thiophiles, des substances activées par la divinylsulfone couplées avec du mercaptoéthanol, la 4-hydroxypyridine, la 3-hydroxypyridine ou la 2-hydroxypyridine ;

des polynucléotides et acides nucléiques naturels et synthétiques, incluant l'ADN, l'ARN, le poly-A, le poly-G, le poly-U, le poly-C et le poly-T ;

des polysaccharides et autres polymères à base de glucide, naturels et synthétiques, incluant la gélose, un alginat, une carraghénine, la gomme de guar, la gomme arabique, la gomme ghatti, la gomme adragante, la gomme karaya, la gomme de caroube, la gomme xanthane, les agaroses, les celluloses, les pectines, les mucines,

les dextranes, les amidons et les héparines ;

des peptides et polypeptides et autres polymères à base d'acides aminés, naturels et synthétiques, incluant les gélatines, les albumines, les hémoglobulines, les immunoglobulines incluant les anticorps poly- et mono-clonaux, les antigènes, la protéine G, les lectines, les glycoprotéines telles que les ovomucoïdes, les protéines fixant la biotine, l'avidine et la streptavidine, les enzymes, les protéases, et les inhibiteurs de protéase ; et des mélanges de ceux-ci.

16. Particules selon la revendication 1, caractérisées en ce que la substance active est liée de façon covalente aux particules d'adsorbant au moyen d'agents d'activation ou de transformation activant ou transformant l'agent conglo-

17. Particules selon la revendication 16, caractérisées en ce que les agents d'activation ou de transformation sont choisis dans un groupe constitué :

des ions bromure de cyanogène, divinylsulfone, épichlorhydrine, bisépoxyranes, dibromopropanol, dialdéhyde glutarique, carbodiimides, anhydrides, hydrazines, periodates, benzoquinones, triazines, tosylates, trésylates et diazonium.

18. Particules selon la revendication 1, caractérisées en ce que la densité est de 0,1 à 15, en particulier de 0,1 à 5, et de préférence de 0,2 à 2.

19. Particules selon la revendication 1, caractérisées en ce que la densité est de 1 à 15, en particulier de 1,1 à 5, et de préférence de 1,1 à 2.

20. Particules selon la revendication 1, caractérisées en ce que la densité est de 0,1 à 1, généralement de 0,2 à 0,95, en particulier de 0,3 à 0,8, et de préférence de 0,5 à 0,75.

21. Particules selon la revendication 1, caractérisées en ce que leur taille est dans une gamme choisie dans le groupe constitué de :

1-10000 μm ,

1-5000 μm ,

1-4000 μm ,

1-3000 μm ,

1-2000 μm ,

1-1000 μm ,

50-500 μm .

22. Particules selon la revendication 21, en particulier pour la purification et la fixation de protéines et d'autres substances de haut poids moléculaire, caractérisées en ce que leur taille est dans une gamme choisie dans les groupes constitués de :

1-2000 μm ,

10-1000 μm ,

50-750 μm ,

100-500 μm , de toute préférence.

23. Procédé de préparation de particules d'adsorbant chromatographique telles que définies dans la revendication 1, caractérisé par les étapes consistant à :

a) mélanger des particules choisies dans le groupe constitué des particules basse densité ayant une densité fournissant une flottation, et des particules haute densité ayant une densité fournissant une sédimentation du conglo-

merat dans le liquide avec au moins un agent conglo-

merant choisi dans le groupe constitué des monomères et polymères organiques naturels et synthétiques ;

et, facultativement, à chauffer ledit mélange ;

b) émulsifier ledit mélange dans un solvant approprié ;

c) consolider ledit agent conglo-

merant par un moyen approprié tel qu'une gélification par chauffage/-refroidissement, une polymérisation d'un monomère ou de mélanges de monomères, et une réticulation non covalente ou covalente ; et à

d) isoler et laver les particules du conglo-

24. Procédé selon la revendication 23, caractérisé en ce que les particules du conglomerat isolées et lavées sont triées selon la taille et l'aptitude à l'écoulement.

25. Procédé selon la revendication 23 ou 24, caractérisé en ce que l'agent conglomerant ou les particules du conglomerat sont activés ou transformés.

26. Procédé de préparation de particules d'adsorbant chromatographique telles que définies dans la revendication 1, caractérisé par les étapes consistant à :

a) mélanger des particules choisies dans le groupe constitué des particules basse densité ayant une densité fournissant une flottation, et des particules haute densité ayant une densité fournissant une sédimentation du conglomerat dans le liquide

avec au moins un agent conglomerant choisi dans le groupe constitué des monomères et polymères naturels et synthétiques ;

et, facultativement, à chauffer ledit mélange ;

b) consolider ledit agent conglomerant par un moyen approprié tel qu'une gélification par chauffage/-refroidissement, une polymérisation d'un monomère ou de mélanges de monomères, et une réticulation non covalente ou covalente ;

c) désintégrer le bloc de conglomerat ; et à

d) isoler et laver les particules du conglomerat consolidé.

27. Procédé selon la revendication 26, caractérisé en ce que les particules du conglomerat isolées et lavées sont triées selon la taille et l'aptitude à l'écoulement.

28. Procédé selon la revendication 26 ou 27, caractérisé en ce que l'agent conglomerant ou les particules du conglomerat sont activés ou transformés.

29. Utilisation de particules d'adsorbant chromatographique selon la revendication 1, en tant que matrice de phase solide dans un réacteur à lit fluidisé.

30. Utilisation selon la revendication 29 dans un mode opératoire choisi dans le groupe constitué :

des modes opératoires chromatographiques employant des colonnes non remplies, notamment la chromatographie liquide, la chromatographie par échange d'ions et la chromatographie d'affinité biospécifique telle que la chromatographie d'immunosorption et de protéine A, et la chromatographie d'affinité spécifique pour un groupe telle que la chromatographie hydrophobe, thiophile, d'un colorant, de lectine et d'un chélate de métal.

31. Utilisation selon la revendication 29 ou 30, caractérisée en ce que le réacteur à lit fluidisé est un réacteur à lit fluidisé à écoulement descendant de liquide comprenant un récipient de réacteur vertical avec une entrée, une sortie, un lit fluidisé desdites particules d'adsorbant chromatographique, et des moyens d'agitation ; lesdits moyens d'agitation étant situés près du lit fluidisé, ou dans celui-ci, près de l'entrée de liquide.

32. Utilisation selon la revendication 31 pour distribuer le liquide dans le lit fluidisé, caractérisée

a) en ce que les particules d'adsorbant chromatographique et le liquide près de l'entrée de liquide sont agités pour diviser le lit fluidisé

i) en une zone turbulente ayant des particules se déplaçant vigoureusement, et

ii) en une zone non turbulente ;

ladite zone non turbulente étant adjacente à ladite zone turbulente ; et

b) en ce que l'étendue de ladite zone turbulente est déterminée par le degré d'agitation, choisi dans une gamme allant

i) d'un degré d'agitation fournissant de la turbulence seulement dans la partie supérieure du lit fluidisé,

ii) jusqu'à un degré d'agitation fournissant de la turbulence des particules au travers du lit fluidisé.

33. Utilisation selon la revendication 29 ou 30, caractérisée en ce que le réacteur à lit fluidisé est un réacteur à lit fluidisé à écoulement ascendant de liquide comprenant un récipient de réacteur vertical avec une entrée, une sortie,

un lit fluidisé desdites particules d'adsorbant chromatographique, et des moyens d'agitation ; lesdits moyens d'agitation étant situés près du lit fluidisé, ou dans celui-ci, près de l'entrée de liquide.

34. Utilisation selon la revendication 33, pour distribuer le liquide dans le lit fluidisé, caractérisée

a) en ce que les particules d'adsorbant chromatographique et le liquide près de l'entrée de liquide sont agités pour diviser le lit fluidisé

i) en une zone turbulente ayant des particules se déplaçant vigoureusement, et

ii) en une zone non turbulente ;

ladite zone non turbulente étant adjacente à ladite zone turbulente ; et

b) en ce que l'étendue de ladite zone turbulente est déterminée par le degré d'agitation, choisi dans une gamme allant

i) d'un degré d'agitation fournissant de la turbulence seulement dans la partie inférieure du lit fluidisé,

ii) jusqu'à un degré d'agitation fournissant de la turbulence des particules au travers du lit fluidisé.

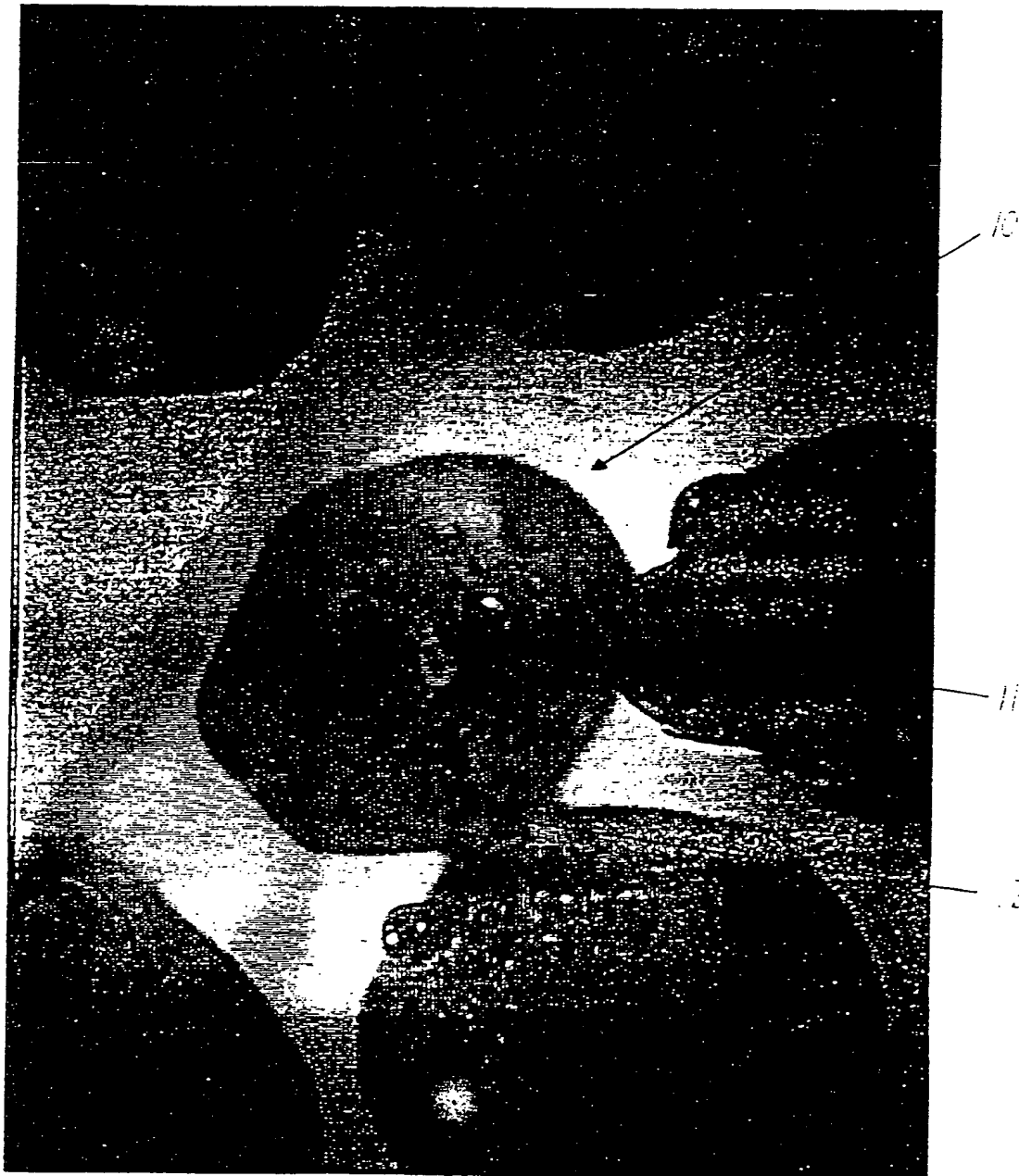


FIG. 1A

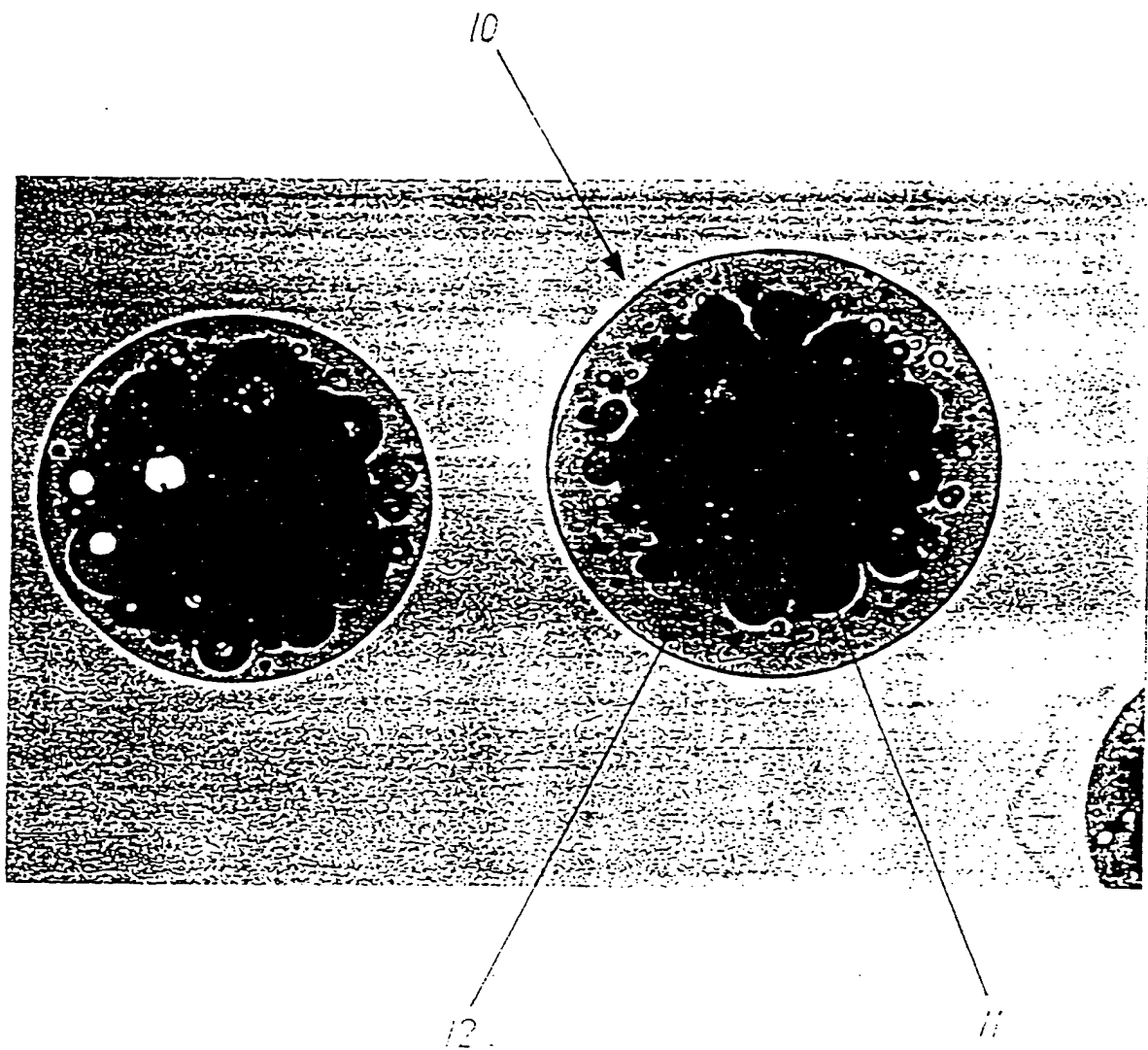


FIG. 1B

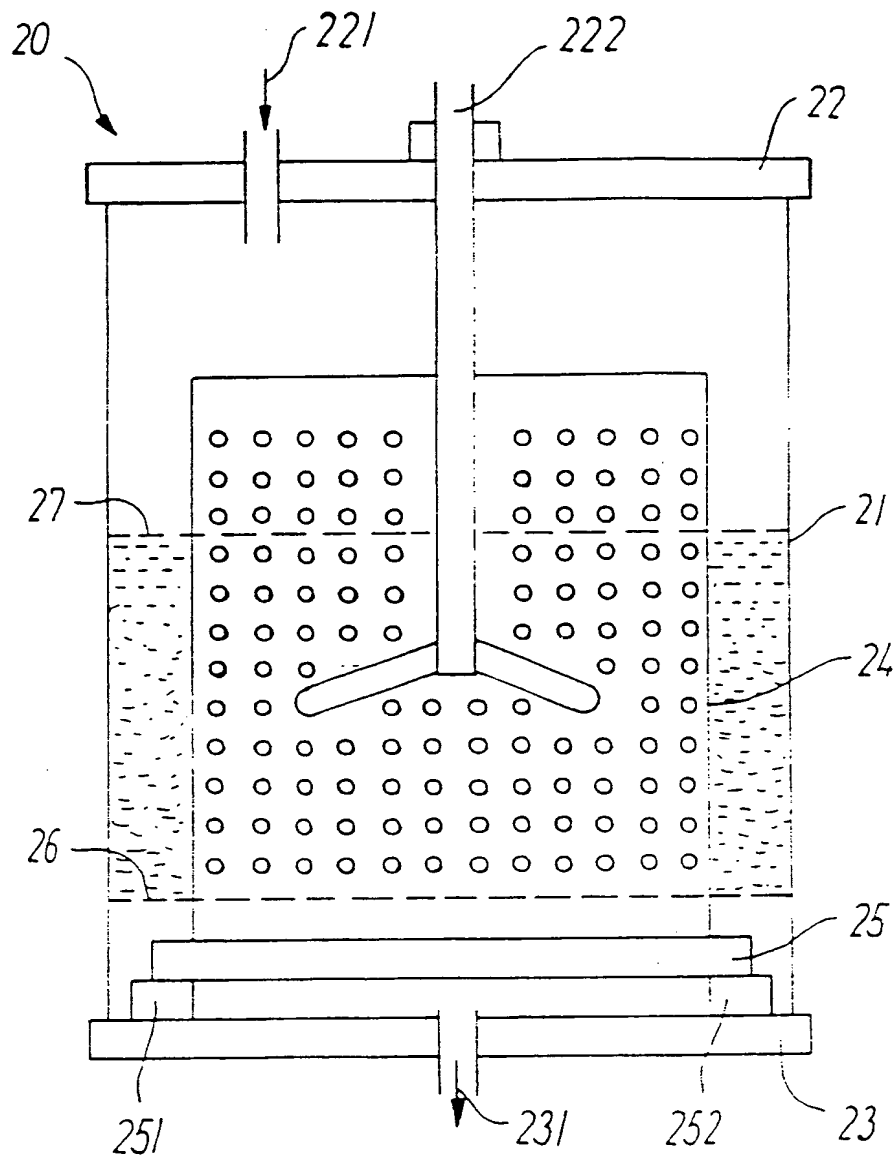


FIG 2

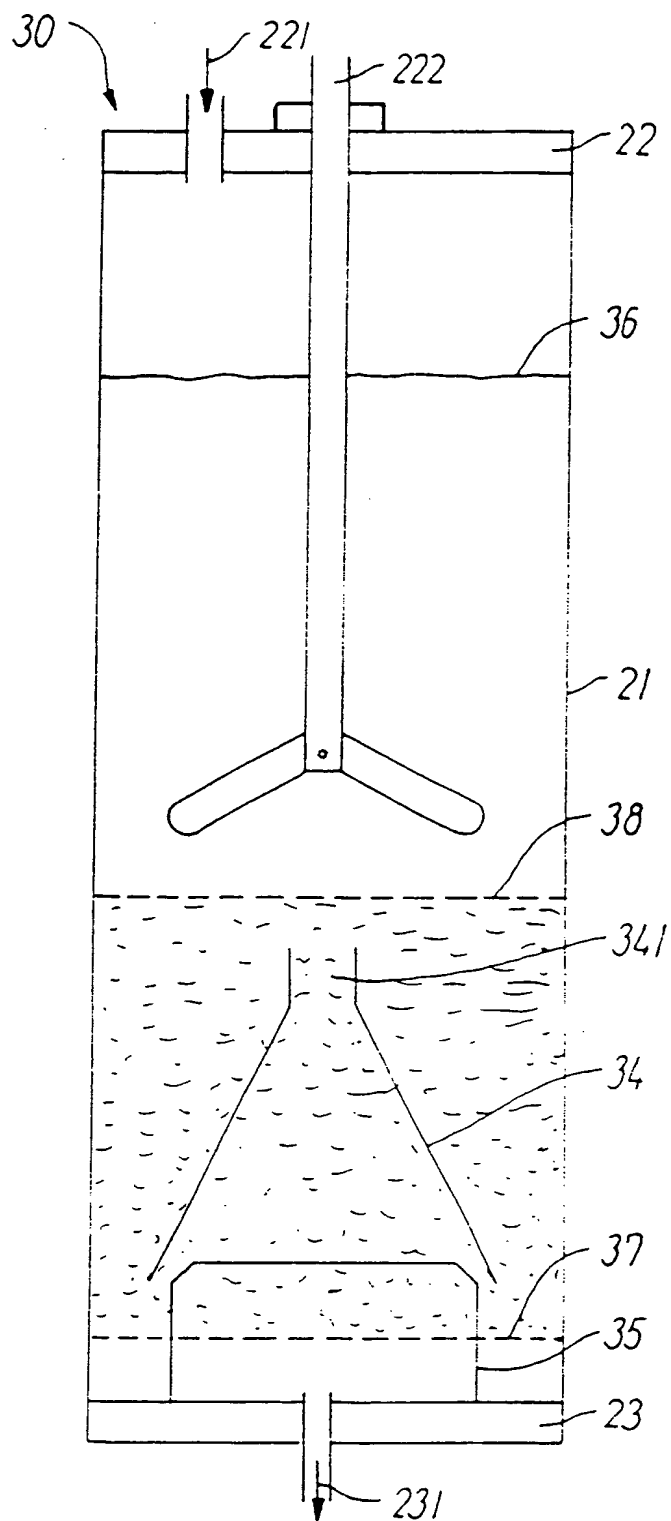


FIG. 3

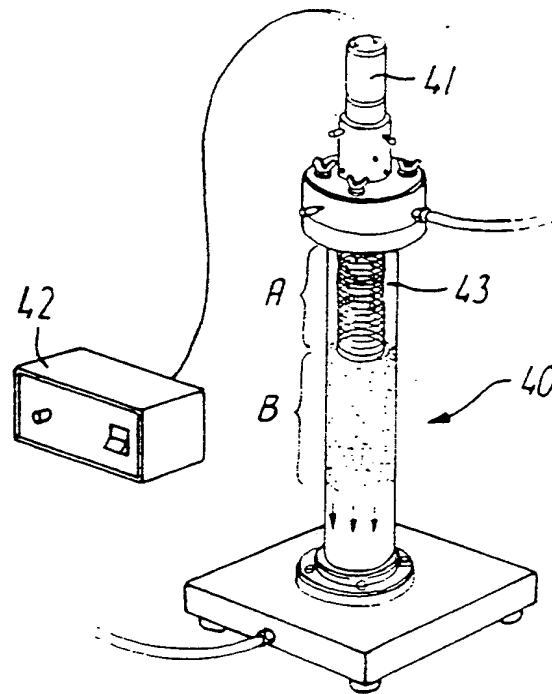


FIG. 4A

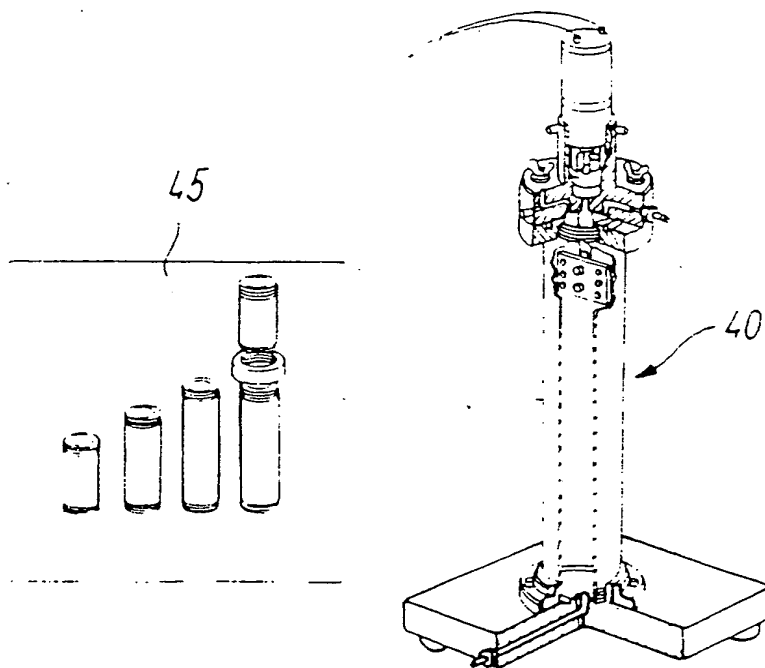


FIG. 4B

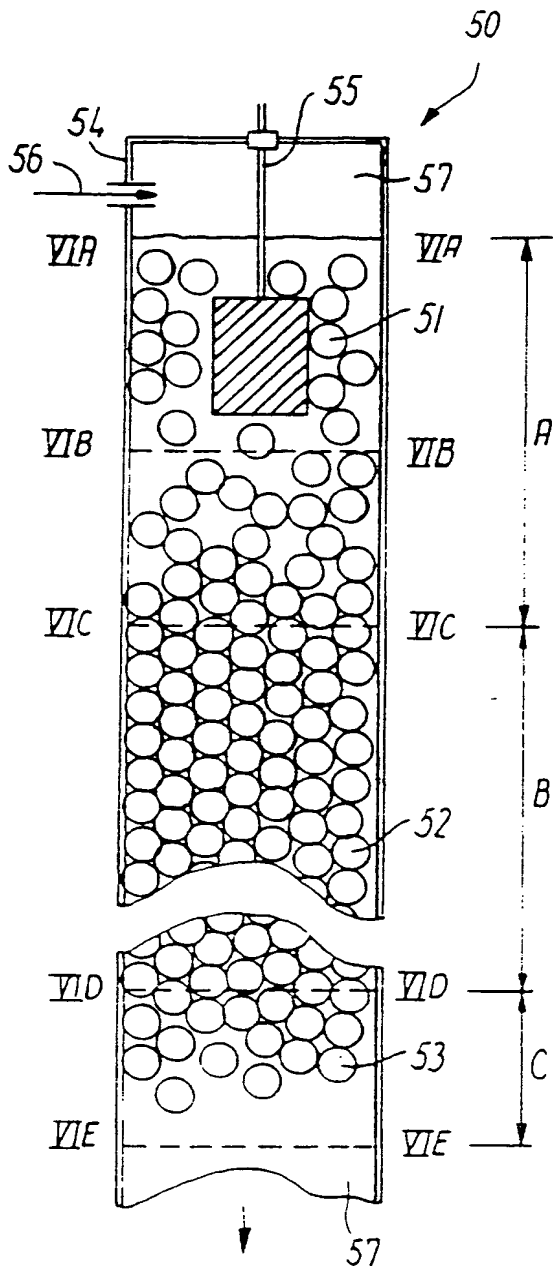


FIG. 5

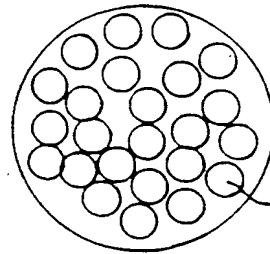


FIG. 6A

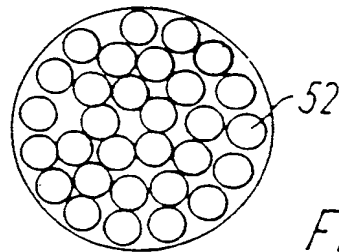


FIG. 6B

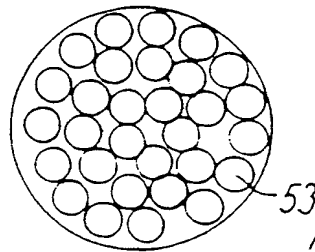


FIG. 6C

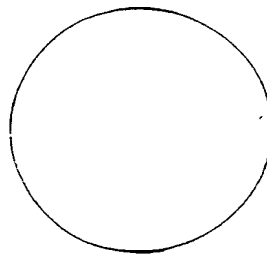


FIG. 6D

